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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

JOINT MEETING

A JOINT Meeting of the Society and the Pharmaceutical Society of Great Britain was held at 7.30 p.m. on Wednesday, November 25th, 1959, at the Pharmaceutical Society, 17 Bloomsbury Square, London, W.C.1. Owing to the absence through illness of Dr. K. R. Capper, B.Pharm., F.P.S., D.I.C., the chair was taken by Dr. D. C. Garratt, F.R.I.C., Chairman of the Analytical Methods Committee of the Society for Analytical Chemistry.

The subject of the meeting was "Methods of Assays of Capsicum, Lonchocarpus and Rauwolfia" and the following papers were presented and discussed: "Determination of Capsaicin in Capsicum and its Preparations," by H. B. Heath, M.B.E., B.Pharm., F.P.S.; "Determination of Rotenone in Lonchocarpus, Derris and their Extracts," by R. F. Phipers, B.Sc., Ph.D.; "Determination of Reserpine-type Alkaloids in Rauwolfia," by C. A. Johnson, B.Sc., B.Pharm., F.P.S., A.R.I.C.

NEW MEMBERS

ORDINARY MEMBERS

Albert Gordon Barker; David James Brindley; Anthony Deuchar Care, M.A. (Cantab.), Ph.D. (Leeds), B.V.M.S. (Edin.), M.R.C.V.S.; Clifford Paul Cole; Ronald Bernard Cooke; John M. Corliss, B.S. (Loyola); Professor Ugo Croatto, Dr.Chem.; Alan Fielder Dewey, B.Sc. (Lond.); Stephen James Hawkes, B.Sc. (Lond.); John Frederick Hinsley, F.I.M.; Michael Anderson Leonard, B.Sc., Ph.D. (Birm.); Alan Marshall, F.P.S.; Robert L. Maute, B.S. (Colorado), M.A. (Houston); Percy John Moore, B.Sc. (Lond.), A.R.I.C.; Ronald Moxon; William Clarke O'Neill, B.A. (T.C.D.), A.R.I.C., M.I.C.I.; Peter Melvin Owens; Derek Julian Podesta; Richard Alexander Hamilton Pool, B.A. (Oxon.); George Harrington Rogers; Dale John Stamp, B.Sc. (Dunelm.); Ronald John Thompson, A.R.I.C.; Albert John Woiwod, B.Sc., Ph.D. (Lond.), A.R.I.C.

JUNIOR MEMBERS

John Alfred Barton; Keith E. Burke, B.S. (Monmouth), M.S. (Iowa); Robert Anthony Feather; William Tompson Gore, B.Sc. (Rhodes); Margaret Jean Hill, B.Sc. (Birm.); Julian Martin Jacobs, B.Sc. (Lond.), A.R.C.S.

NORTH OF ENGLAND SECTION AND PHYSICAL METHODS GROUP

A JOINT Meeting of the North of England Section and the Physical Methods Group of the Society and the Modern Methods of Analysis Group of the Sheffield Metallurgical Association was held at 7 p.m. on Tuesday, October 13th, 1959, in the Conference Room of the British Iron and Steel Research Association, Hoyle Street, Sheffield 3. The Chair was taken by the Chairman of the Physical Methods Group, Mr. R. A. C. Isbell, A.Inst.P.

The subject of the meeting was "Modern Methods of Titration" and the following papers were presented and discussed: "Coulometry in the Analysis of Metals," by G. Mattock, B.Sc., Ph.D., A.R.I.C. (see summary below); "High Frequency Titration," by E. S. Lane, B.Sc., Ph.D., F.R.I.C.

Coulometry in the Analysis of Metals

Dr. G. Mattock said that coulometric analysis depended on the application of Faraday's law that passing 96,493 absolute coulombs liberated one gram-equivalent of ions or atoms at an electrode. Two types of process could be used—

(*a*) primary processes, where the ion or molecule reacted directly at the electrode (as in direct reduction of a metal ion at a cathode); or

(*b*) secondary processes, where a reagent was generated at an electrode to react quantitatively with (*i.e.*, to titrate) the substance of interest: these were important where the latter did not undergo direct electrode reaction, or where polymerisation might occur.

In either case, measurement of the number of coulombs required for the completion of the process gave the number of gram-equivalents of substance present, provided 100 per cent. current efficiency was maintained.

There were two methods of carrying out coulometric analyses: at a controlled potential (primary process), where current was measured as a function of time, giving an exponential curve (the area under which gave the number of coulombs), and by titration (secondary process). Only the latter was considered in this lecture.

There was no fundamentally new principle involved in the titration process, which in coulometry involved the electrogeneration of titrant to the titration end-point. The latter was identified potentiometrically, amperometrically or colorimetrically: and Dr. Mattock discussed the relative merits of these techniques. The types of titration could be those used in ordinary volumetric practice, *i.e.*, pH, redox, complexometric, or precipitation: the only requirements were a suitably chosen titrating ion and, of course, full current efficiency. Certain outstanding advantages resulted from the use of coulometry—

(*i*) the high conversion factor from electrical to chemical units, coupled with the accuracy with which electrical measurements could be made, enabled very low concentrations to be determined;

(*ii*) no reagent standardisation was required, and no storage problem for unstable titrants existed; and

(*iii*) the scheme was readily adaptable to automatic operation.

The apparatus used for secondary titrations often involved the passing of a constant current, with time measurement to give the number of coulombs; alternatively, an integrating motor could be used, which gave a direct measure of the number of coulombs.

The author discussed an automatic instrument, developed from one described by L. G. Smythe (*Analyst*, 1957, **82**, 228), to illustrate the principles. This instrument incorporated an integrating motor with a dekatron display reading directly in micro-equivalents. Desirable features of cell design were also indicated.

A number of examples of titrations of interest in metallurgical analysis were considered, including redox titration of metals (such as, *e.g.*, electrogeneration of Fe^{2+} to determine chromium, vanadium or manganese, and electrogeneration of ferrocyanide to determine zinc), and generation of EDTA for titration of various metals, with potentiometric end-point detection.

A Joint Meeting of the North of England Section and the Physical Methods Group of the Society and the Newcastle upon Tyne and North East Coast Section of the Royal Institute of Chemistry was held at 6.30 p.m. on Tuesday, October 27th, 1959, in Chemistry Lecture Theatre, 2, King's College, Newcastle upon Tyne, 1. The Chair was taken by the President of the Society, Mr. R. C. Chirnside, F.R.I.C.

The subject of the meeting was "X-ray Fluorescence" and the following papers were presented and discussed: "Instrumental Developments in X-ray Fluorescence Spectroscopy," by J. R. Stansfield, M.A., F.Inst.P.; "Some Applications of X-ray Spectrography," by H. I. Shalgosky, B.Sc., A.R.I.C.

The meeting was preceded at 2.30 p.m. by a visit to Winthrop Laboratories Ltd., Fawdon.

NORTH OF ENGLAND SECTION

An Ordinary Meeting of the Section was held at 7.30 p.m., on Friday, October 30th, 1959, at the Metropole Hotel, Stockton-on-Tees. The Chair was taken by the Chairman of the Section, Dr. J. R. Edisbury.

The following paper was presented and discussed: "The Changing Aspects of Chemical Analysis," by H. N. Wilson, F.R.I.C.

The meeting was preceded at 2.15 p.m. by a visit to the Analytical Laboratories, Research Department, I.C.I. Billingham Division.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 5 p.m. on Friday, October 30th, 1959, in the Chemistry Department, Queen's College, Dundee. The Chair was taken by the Chairman of the Section, Mr. A. N. Harrow, A.H.-W.C., F.R.I.C.

The following paper was presented and discussed: "Complexones, with Special Reference to EDTA," by H. J. Cluley, M.Sc., Ph.D., F.R.I.C.

WESTERN SECTION

A JOINT Meeting of the Western Section with the Mid-Southern Counties Section of the Royal Institute of Chemistry was held at 7.45 p.m. on Friday, October 30th, 1959, at the Cathedral Hotel, Salisbury. The Chair was taken by the Chairman of the Mid-Southern Counties Section of the Royal Institute of Chemistry, Dr. J. M. Wright, B.Sc., A.R.C.S., D.I.C.

A lecture on "Chemical Analysis and Clinical Diagnosis" was given by Professor Sir E. Charles Dodds, M.V.O., D.Sc., Ph.D., M.D., Hon.Sc.D., F.R.C.P., F.R.I.C., F.R.S.E., F.R.S.

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 7 p.m. on Tuesday, October 27th, 1959, at the Nottingham and District Technical College, Burton Street, Nottingham. The Chair was taken by the Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

A discussion on "The Purity of Analytical Reagents" was opened by J. T. Yardley, B.Sc., F.R.I.C.

MICROCHEMISTRY GROUP

THE twenty-first London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, October 21st, 1959, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Vice-Chairman of the Group, Mr. C. Whalley, B.Sc., F.R.I.C.

A discussion on "The Dumas Determination of Nitrogen" was opened by S. Bance, B.Sc., F.R.I.C., and Miss M. Corner, B.Sc., F.R.I.C.

Zone Melting, with Some Comments on its Analytical Potentialities

A Review

By E. F. G. HERINGTON

(*Pure Compounds Group, National Chemical Laboratory, Teddington, Middlesex*)

SUMMARY OF CONTENTS

ZONE melting is the name of a family of techniques that includes zone refining, a method for the preparation of substances in a high state of purity, and zone levelling, a method for the preparation of uniform solid mixtures. Zone refining[1] provides the analyst with a means of obtaining reference specimens for the calibration of physical analytical instruments. Highly purified standard samples[2] are essential for carrying out quantitative analysis by gas - liquid chromatography or by visible, ultra-violet, infra-red or mass spectrometry if the accuracy of the final analytical result is not to be limited by the purity of the specimens used to standardise the equipment. Further, there is a continuing interest in freezing-point and melting-point methods for establishing the purity of commercial samples (*e.g.*, lindane). In such work a pure sample is required for use in plotting the melting-point - composition curve in the region of 100 per cent. purity, and zone refining can frequently be employed to obtain suitably pure material. Certain volumetric standards can also be prepared by zone refining[3,4]; see, for example, an advertisement[3] describing the preparation of benzoic acid "Purified for Volumetric Standardisation." Analysts may also find zone refining useful for concentrating impurities in a specimen before applying suitable qualitative tests for the impurities, but the theoretical discussion presented later indicates that zone refining is unlikely to be a useful method for concentrating impurities before accurate quantitative analysis.

Since zone levelling affords a method for the preparation of solid samples containing a uniform distribution of one substance in another, it should be of interest to analysts wishing to obtain uniform powders for the calibration of instruments, such as those used in emission spectroscopy.

The segregation of impurities that occurs during the cooling of a metal ingot has been known for a long time, but it was not until 1952 that Pfann[1] realised that use could be made of this phenomenon in the purification of substances. Pfann[5] reports that he conceived the idea of zone refining "while napping at my desk after lunch." His investigations received impetus from the demand for extremely pure germanium and he devised the method of slowly passing a rod of this element through a short furnace so that a molten zone traversed the length of the specimen. In this technique the impurities tend to be carried forward in the molten zone and purer germanium recrystallises from the melt. Zone refining can be extremely efficient; for example, the impurity concentration in the major part of a germanium rod may be reduced to 10^{-9} times that originally present if the zone is passed

several times. Later, zone melting was shown to be applicable to a wide range of metals, inorganic compounds and organic compounds. Numerous articles on the subject have now appeared; Pfann has written a book,[6] the Royal Institute of Chemistry has issued a monograph,[7] reviews have been published[8,9,10] and popular accounts have been prepared.[11,12] Pfann's book[6] will be cited frequently in this review to avoid an undue number of references.

In addition to these reviews several hundred papers on zone melting have been published. No attempt will be made to summarise them all, but sufficient references will be cited to enable an analyst to make an informed choice of the most promising method for preparing a pure reference sample or making a uniform specimen. The next section, on theory, should provide an introduction to the necessary basic concepts.

Theory

Zone refining—

This process depends upon the difference in solubility of an impurity in the liquid and in the solid main component. Throughout the discussion it will be assumed that there is perfect mixing in the liquid, but that no mixing (*i.e.*, no diffusion) takes place within the solid. Since the behaviour of a mixture when submitted to zone refining can be explained in terms of the liquidus and solidus curves for the mixture, the properties of some of these curves will now be discussed. Figs. 1 (*a*), 2 (*a*) and 3 (*a*) show such curves for three types of systems. In all these diagrams component A is regarded as the main component, whereas a small amount of component B is regarded as an impurity.

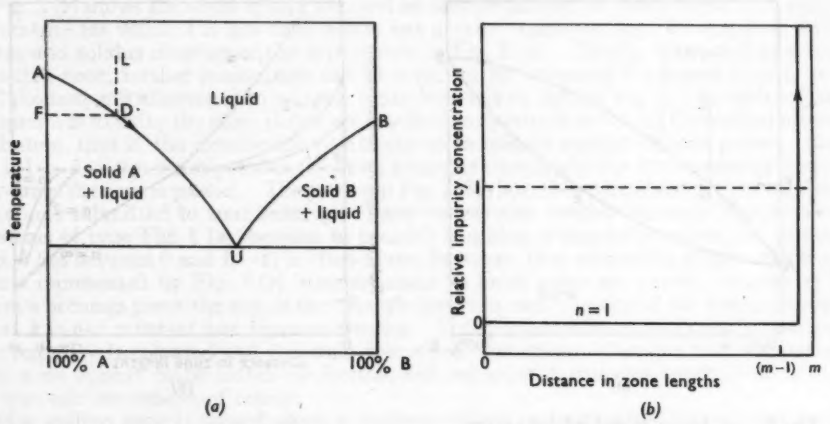

Fig. 1. (*a*) Liquidus and solidus curves
(*b*) Distribution of impurity after one passage of the zone

Fig. 1 (*a*) shows the liquidus and solidus curves for a system exhibiting a simple eutectic. Pure component A begins to separate from such a mixture when a liquid of composition L is cooled to temperature F. On further cooling pure solid A continues to separate and the liquid temperature and composition follow the curve DU in the direction shown by the arrowhead. When the temperature and composition of the liquid reach the point U, solid of composition U separates until the whole charge has solidified. The final material thus consists of solid A encrusted with eutectic of composition U.

Fig. 2 (*a*) shows the liquidus and solidus curves for a system forming a continuous series of solid solutions. If a liquid mixture of this type having composition L is cooled, the solid that first crystallises out will have composition E. If more heat is extracted from the system the temperature and composition of the solid will move in the direction shown by the arrowhead on the lower curve. Thus the layers of solid deposited will have compositions corresponding to successive points on the lower curve. Finally, when the temperature reaches the freezing-point of pure component B, all the material will have solidified. In this system pure A is not deposited at any stage.

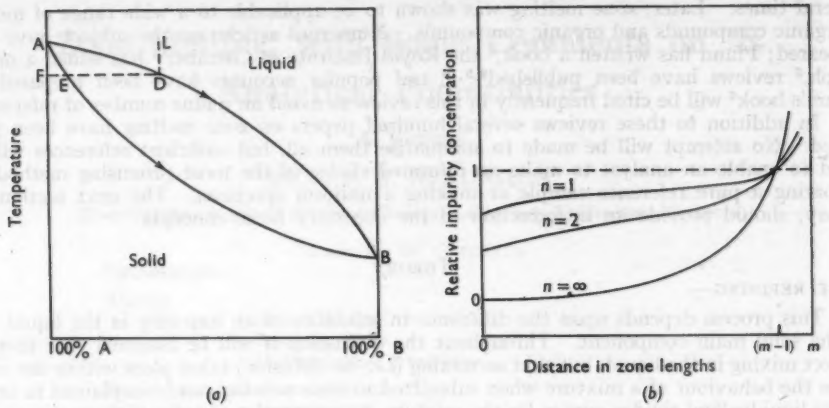

Fig. 2. (*a*) Liquidus and solidus curves
(*b*) Distribution of impurities after one, two and an infinite number of zone passes

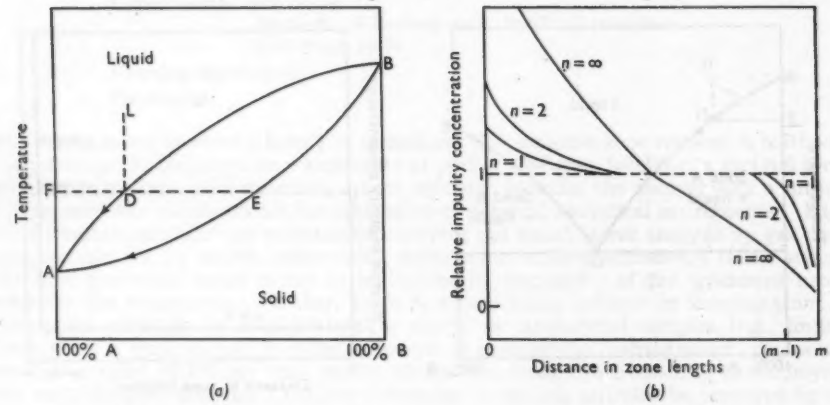

Fig. 3. (*a*) Liquidus and solidus curves
(*b*) Distribution of impurities after one, two and an infinite number of zone passes

Fig. 3 (*a*) shows the liquidus and solidus curves for a system in which the main component A forms mixed crystals with the higher-melting impurity B. The solid deposited in this system is always richer in component B than the liquid from which it has come.

In discussing zone-refining experiments it is convenient to define an ideal distribution coefficient k_i as the concentration of B in the solid divided by the concentration of B in the liquid in equilibrium with that solid. Thus in Figs. 2 (*a*) and 3 (*a*) the ideal distribution coefficient k_i equals FE/FD, where FE and FD are the lengths of the lines on the figures. In Fig. 2 (*a*) the value of k_i lies between 0 and 1, in Fig. 3 (*a*) the value of k_i is greater than 1 and in Fig. 1 (*a*) the value of k_i is 0. A quantity of more importance in zone melting is k, the effective distribution coefficient, which equals the real concentration of B in the solid that has just been deposited divided by the real concentration of B in the liquid from which the solid has just separated. In general, k is nearer to unity than k_i when the rate of freezing is not infinitely slow.

Consider now the zone refining of a solid mixture of type Fig. 1 (*a*) and initially of uniform composition L. If this mixture is placed in the zone-refining apparatus shown diagrammatically in Fig. 4 then theoretically one passage of the zone should suffice to produce 100 per

cent. pure A throughout the bar except for a portion near the end, which will have composition U. The theoretical distribution of impurity after one zone passage ($n = 1$) is shown in Fig. 1 (*b*) for a mixture initially containing a low concentration of B. In Fig. 1 (*b*) and also in Figs. 2 (*b*) and 3 (*b*), the relative impurity concentration of unity represents the initial uniform concentration and the distance along the rod is expressed in terms of zone length. The ideal separation shown in Fig. 1 (*b*) presupposes perfect crystal growth with no entrainment of liquid during zone refining; these conditions are rarely achieved in practice. However, a solution of sodium chloride in water has been reported[13] as conforming to these conditions because over 99·9 per cent. of salt was removed from a solution containing 20 g per litre by a single pass of the heater. However, the zone refining of a mixture with a phase diagram of the form shown in Fig. 1 (*a*) usually gives results similar to those obtained from a mixture of the type represented by Fig. 2 (*a*).

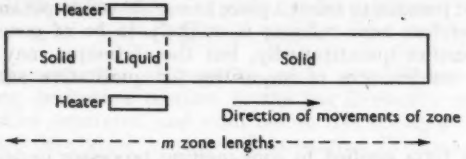

Fig. 4. Diagramatic representation of a zone-refining apparatus

Fig. 2 (*b*) shows the result of one, two and an infinite number of passes in the zone refining of a mixture for which k is less than unity, but greater than zero, *e.g.*, for a system with a liquidus and solidus diagram of the type shown in Fig. 2 (*a*). Clearly, after each passage of the molten zone, further purification can be achieved by returning the heater rapidly to its initial position and allowing it to traverse again from left to right in Fig. 4. In such a system the nearer k is to unity the more passes are required to approximate to the theoretical ultimate distribution, that is, the distribution equivalent to an infinite number of zone passes. Moreover, if $1 > k > 0$ it is not possible to obtain a sample completely free from impurity however many times the zone is passed. The curves in Fig. 2 (*b*) show the commonest type of behaviour of mixtures submitted to zone refining. These curves also roughly represent the behaviour of systems of type Fig. 1 (*a*), because in practice trapping of impurity occurs, *i.e.*, although k_i is 0, k lies between 0 and 1. It is often found, however, that separation of impurity from a mixture represented by Fig. 1 (*a*) becomes easier as more zones are passed, because as the specimen becomes purer the size of the crystals increases and trapping of the liquid decreases, so that k is not constant but becomes smaller. This phenomenon is particularly noticeable if the impurity is coloured and the main component colourless; often the first few passages of the zone appear to be rather ineffectual, but subsequent passages bring about a rapid and dramatic movement of colour.

If a molten zone is passed along a uniform rod of material with liquidus and solidus curves of the type Fig. 3 (*a*), initially of composition L, the impurity will move in the opposite direction to the zone because $k>1$ and successive passes bring about the concentration changes shown in Fig. 3 (*b*). There is an essential difference between systems where k is greater than 1 and where k is less than 1, as the following considerations show. Imagine a zone in the middle of its traverse during the purification of a compound when k is less than unity, any molecule of impurity that is picked up at the melting interface may be carried to the end of the rod during this zone traverse. If k is greater than 1, however, a molecule during one zone passage cannot make a movement greater than one zone length towards the end from which the zone has come. Hence when $k>1$ the number of passes required to bring about a considerable concentration of impurity near the front of the rod must be greater than the number of zone lengths in the rod of material. Thus in general the movement of impurity is much slower when $k>1$ than in a system for which $k<1$. If some of the impurities in a specimen exhibit values of k greater than 1 and others less than 1 it is desirable to use a longer rod of material than usual, and at the end of the experiment the centre section will be the purest.

Mixtures may exhibit types of liquidus and solidus curves other than those shown in Figs. 1 (*a*), 2 (*a*) and 3 (*a*), but provided the material is initially rich in one component then the behaviour on zone refining can be summarised in the statement—

If the impurity depresses the melting-point of the main component it will tend to move in the same direction as the zone, but if the impurity raises the melting-point it will tend to move in the opposite direction.

If the original mixture is of the type shown in Fig. 1 (*a*) and if the composition is slightly to the left of U, then after many passages of the zone the rod will consist of a small amount of pure A and a large amount of the eutectic of composition U, *i.e.*, the separation of the components is far from complete. Thus zone refining is a useful complementary technique to fractional distillation when both can be used, because distillation is most useful in the middle composition range[14] and zone refining is most efficacious when one component is in considerable excess, and this is just the condition where the application of distillation is least rewarding.

Since Fig. 2 (*b*) is typical of the behaviour exhibited by most systems submitted to zone refining, it is usually not possible to select a place to cut the rod to obtain a specimen containing all the impurity. Therefore zone refining is unlikely to be of general use to the analyst for concentrating impurities quantitatively, but the technique may provide a convenient method for obtaining concentrates of impurities for qualitative analysis.

ZONE LEVELLING—

Zone levelling is a term applied to zone-melting processes undertaken with the object of achieving as uniform a concentration as possible over as great a length of the rod as possible. Several procedures can be adopted to attain this end provided the solidus - liquidus diagram is not of the type shown in Fig. 1 (*a*).

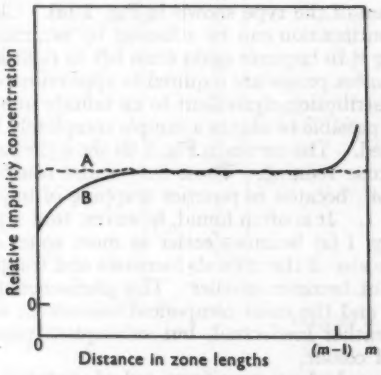

Fig. 5. Zone levelling when k is near unity: curve A, initial composition; curve B, composition after one zone passage

If k is near unity a single passage of a zone through a rod of material of fairly uniform composition will yield a central portion of even more uniform composition (see, for example, Fig. 5). A method of wide applicability is to pass the heater repeatedly at constant speed in alternate directions. This procedure, which brings about zone levelling in all parts of the rod except the part to freeze last, can be used when the value of k is not known, though it is then difficult to produce a rod of selected composition.

If k is known it is often possible to arrange[6] that the solute concentration in the first zone length has the value C_0/k, where C_0 is the average initial concentration of the dilute component in the remainder of the bar. If a molten zone is now moved along the bar the amounts of impurity entering and leaving the zone are equal, so that the concentration in the bar after passage of the zone is uniform and equal to C_0, except for the last zone.

Other methods of bringing about zone levelling have been described.[6] Zone levelling should be generally useful to the analyst for preparing reference samples of uniform composition.

MATHEMATICAL TREATMENT OF ZONE REFINING—

In considering the mathematical theory it is usual to discuss first the behaviour of a bar of material containing a small amount of impurity undergoing normal freezing. The sample is assumed to be liquid at the beginning of the experiment, and then freezing is allowed to take place from one end. The process is therefore sometimes called directional freezing, and this type of freezing takes place in the last zone length of a zone-refining experiment.

If the following simplifying assumptions are made—

(*a*) that complete mixing of the liquid is achieved at all stages,

(*b*) that no diffusion takes place in the solid and

(*c*) that the effective distribution coefficient is constant,

then it can be shown[15,16,17,18,19] that C, the concentration of impurity at a point where a fraction, g, of the original liquid has frozen is given by the equation—

$$C = kC_0 (1 - g)^{k-1} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where C_0 is the mean concentration of impurity in the rod. This equation at the best is only approximately true, because a position in the bar is usually reached where material of the eutectic composition separates, and even before this occurs k has often ceased to be constant and has become dependent upon g. This normal directional-freezing process has been used for purification purposes; thus Schwab and Wichers[20] prepared benzoic acid of purity 99·997 mole per cent. in 40 per cent. yield from material of 99·91 mole per cent. purity by three re-crystallisations, rejecting impure portions at each step. Recently, Dickinson and Eaborn[21] have re-examined directional freezing, but their claim that it is at least as effective as the more complex zone-refining procedures must be treated with reservation. When k equals 0 both processes are equally effective. When k is near 1, however, zone refining is superior, because it is easier to apply and because the yield of highly purified material is greater; the yield of highly purified material from repeated directional-freezing experiments is very low if k is near unity.

Mathematical treatments of zone refining have been carried out by using the simplifying assumptions given below, which are additional to conditions (*a*), (*b*) and (*c*) presented above—

(*d*) that the concentration of impurity, C_0, is initially uniform and

(*e*) that a constant cross-section and constant length of zone is used.

The mathematical analyses of the effects of a single passage of a zone and of an infinite number of zone passes when conditions (*a*) to (*e*) are satisfied were presented by Pfann.[19] The study of a single passage of a zone leads to the equation—

$$C = C_0 [1 - (1 - k)e^{-kx/l}] \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where l is the length of the zone and x is the distance along the rod. Equation (2) is not applicable to the last zone length, to which equation (1) usually applies. An approximation[6] to the impurity distribution after a large number of passes is given by the equation—

$$C = Ae^{Bx} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

A and B are related to other quantities by the equations—

$$k = Bl/(e^{Bl} - 1) \; .. \quad .. \quad .. \quad .. \quad .. \quad (4a)$$

$$A = C_0 BL/(e^{BL} - 1) \quad .. \quad .. \quad .. \quad .. \quad (4b)$$

where L denotes the length of the bar. Calculation of the distribution of impurity for a number of passes of the zone, when n is greater than 1 but less than infinity, is more difficult. The results of detailed calculation and methods for deriving the necessary equations have been collected by Pfann.[6] Burris, Stockman and Dillon[22] have also published a convenient collection of curves showing impurity concentration plotted against the position in the bar for different values of k and for different numbers of passes. Such curves are valuable when k is known and when a binary mixture is being dealt with, but if an analyst wishes to purify a material suspected to contain many impurities with unknown values of k then these curves are of little use. However, these calculations can be employed for choosing the best procedure for any particular circumstances. The detailed calculations show that there is an advantage in using a relatively long zone in the early stages to bring about a rapid movement of impurity and a short zone in the later stages to bring about a greater degree of purification. Ratios

of bar length to zone length of 10 or greater in the later stages should permit a good final purification to be achieved.

The number of passages of the zone necessary to produce a concentration distribution approximate to the ultimate one depends upon the value of k and on the ratio of bar length to zone length. Calculation shows that 20 passes will bring about a separation near to that ultimately obtainable if the value of k is 0·1 and if the ratio of bar length to zone length is 10. Many hundreds of passes may be needed to effect good separation if k is greater than 0·5. In the chemical laboratory it is usually reasonable to try between 10 and 20 passes if nothing is known about the system, but if the separation is found to be an easy one (*i.e.*, if k is small) fewer passages of the zone will suffice.

When the maximum separation possible with a particular bar length and zone length has been achieved no advantage is gained by removing an impure section and continuing the process unless the ratio L/l can be increased to at least its original value. This increase in ratio can be brought about either by making the sample into a longer rod or by reducing the length of the zone.

Some practical considerations

Measurement of k—

A knowledge of k can be used to calculate the distribution of a single impurity at any stage during zone-refining experiments. Various methods have been described[6] for determining the value of this constant, but the simplest procedure is to allow a cylinder of molten material to crystallise from one end at the same rate and with similar mixing in the liquid as will be used in the zone-refining experiments. Portions taken from different sections along the bar are analysed and k is determined by drawing the best straight line through values of $\log(C/C_0)$ plotted against $\log_{10}(1-g)$. From equation (1) it can be seen that the slope of this line equals $(k-1)$ and the intercept on the vertical axis, where the value of $\log_{10}(1-g)$ is zero, equals $\log_{10}k$.

Speed of zone movement—

In general the rate of diffusion of impurity in the solid is small, so that there is considerable advantage in moving the zone slowly in order to allow time for the impurities in the liquid to diffuse away from the advancing solid face. On the other hand, it is desirable to carry out the process rapidly in order to obtain the product as quickly as possible. Pfann[6] has discussed methods of computing the optimum rate when k is known and when the value of δ, the thickness of the diffusion layer, can be estimated, but such calculations are not readily carried out when more than one impurity is present. When only a single sample of a pure material is required and it is not feasible to examine the effects of all the variables, rates of zone movement of 6 inches per hour for metals and semiconductors[6] and of 1 to 1·5 inches per hour for organic compounds[23] may be employed in exploratory experiments.

Volume change and material transfer—

Melting is usually accompanied by a volume change, and this leads to material transfer in zone refining if the substance is treated in a horizontal open boat.[6] Contraction on melting causes material to be moved in the direction of the zone, expansion causes transport in the reverse direction. Material transport of a substance in an open boat can be prevented by tilting the container to the correct angle.[6] If a vertical tube is used the heater must be started at the top if the solid expands on melting and at the bottom if it contracts. In a material that expands on melting, spaces appear at the bottom of the vertical tube as zone refining proceeds and material is transported upwards at each zone pass until a steady condition is reached, in which bubbles rise at each passage of the zone and pass out of the material at the top of the tube at the same rate as new spaces are created at the bottom.

Zone length—

Ideally the zone length should be extremely short in later stages of purification, but in reality the smallest zone that can be achieved experimentally may be longer than that suggested by theory. A rod of small diameter may have to be used to keep the zone short if the material is a poor heat conductor. Thus, for example, tubes of more than 1·5 inches diameter heated from the outside cannot usually be employed for zone refining organic compounds that are poor heat conductors.

Experimental Techniques

Methods of Heating and Choice of Container—

The range of materials that have been treated by zone refining is large and no single piece of apparatus is suitable for treating all substances. Parr[7] has presented a diagram showing the temperature ranges covered by various methods of heating. To attain the highest temperatures (*e.g.*, 3500° C) solar energy may be concentrated by means of reflection, whereas temperatures up to 2000° C can be reached by the use of radiant energy from electric heaters.[24] Bombardment by a focused stream of electrons accelerated by a few kilovolts can be employed to produce a narrow liquid zone[6,25,26] in the temperature range 1000° to 3200° C. Radio-frequency heating can be used for treating some elements melting in the temperature range 0° to 2500° C. Dielectric heating will melt some substances in the range 0° to 500° C, but most organic compounds cannot be heated in this way because they do not exhibit a suitable dielectric loss. Gas flames may be used to produce temperatures up to 500° C and in a simple apparatus of this type the low-melting metal undergoing purification may be contained in a horizontal boat enclosed either in an evacuated tube or in a tube filled with inert gas. Before starting the experiment it may be necessary to remove an oxide film, and it is sometimes advantageous to leave a seed crystal unmelted at the beginning of the bar. The molten zone may spread to a marked degree at the ends of the bar if the material is a good conductor, and it is then necessary either to cool the ends of the bar or to reduce automatically the supply of heat when the zone approaches the end of the bar.[27] Low-melting organic compounds (*e.g.*, *m*-cresol, m.p. 12° C) can be zone refined in an apparatus built within a refrigerator, and even lower-melting materials (*e.g.*, *m*-xylene, m.p. −56° C) can be dealt with in an apparatus enclosed within a space cooled by solid carbon dioxide.[28] Alternatively, the material may be contained in the annular space between two tubes, the inner space being occupied by a heater and the outer tube being immersed in a vessel containing a suitable refrigerant.[29]

Sometimes it is essential to avoid all contamination arising from the container, and it may then be possible to use a floating-zone technique.[6,24,30,31,32,33,34,35] In this method a rod of the material is clamped at the ends and a zone formed by suitable means is supported by surface tension, which is sometimes supplemented by other forces, such as those produced by electromagnetic fields. Induction heating or electron bombardment[6,25,26] may be used to produce a molten zone in an apparatus of this type.

For materials of low surface tension (*i.e.*, most organic compounds) it is necessary to employ a vertical column rather than a horizontal boat, because when the surface tension is small the liquid zone in the latter tends to flow back under the freshly solidified material and contaminate the solid.

Apparatus for treating 1 kg of organic material, in which a simple bobbin electric heater automatically returned to the top for a fresh cycle of operations is used, has been described.[23] The main difficulties with this apparatus arise from occasional breakages, but these can be avoided if a metal tube is used. The position and size of the zone may then be established by using a movable thermocouple in a narrow pocket situated along the axis of the tube.

Small samples from 0·1 to 10 g may be treated in an apparatus employing an ellipsoidal reflector to concentrate the energy from the bulb of a projection lamp on to the sample contained in a small tube and moved by an electric-clock motor.[36] Samples of a few milligrams cannot be manipulated successfully in narrow glass tubes because small gas bubbles usually appear and sub-divide the sample, but this difficulty has been overcome in an apparatus described by Hesse and Schildknecht[37] in which the samples are inserted in a scratch along one side of a glass rod.

Attempts have been made (*e.g.*, see Pfann[38]) to work with large amounts of material by conducting zone refining as a continuous process, but in most reported experiments purification has been carried out batchwise. Time can usually be saved by passing several zones simultaneously in a batch experiment, but unfortunately only one zone can be passed in each run of a floating-zone experiment.

To obtain efficient separation it is essential to ensure good mixing within the molten zone. If a vertical tube is employed and if it is possible to arrange for the heater to travel downwards (*i.e.*, if the solid expands on melting), convection currents usually produce sufficient mixing. Pfann's book[6] should be consulted for other methods of stirring the liquid.

EFFICIENCY TESTS—

The efficacy of a zone-refining apparatus for purifying a particular element may be studied by using a suitable radioactive tracer. For example, by adding traces of silver-110 and antimony-124 to bismuth it has been shown that these impurities move in opposite directions and the optimum speed of zone movement has been established.[39] Such tracers can give quantitative information on the removal of a specific impurity from a specific element, but the general efficiency of a design of zone-refining apparatus for treating organic substances can be examined by using a dye or a fluorescent material soluble in the liquid but insoluble in the solid main component. Thus a solution of 0·03 per cent. of indulin,[40] a blue dye, or of 0·2 per cent. of anthracene,[41] a material fluorescent under ultra-violet light, in naphthalene have been used to study the efficiency of apparatus design. To indicate the degree of separation that can be achieved it may be noted that the passage of eight zones reduced the anthracene content in half the charge to 1/10,000th part of its original value.

UNUSUAL SEPARATIONS

If the impurity is much more volatile than the main component it may not be possible to obtain good separations by zone refining in a horizontal evacuated tube, owing to redistribution of the impurity as vapour through the space above the charge. Van den Boomgaard[42] has examined theoretically and experimentally the behaviour of volatile components in zone levelling. In practice, improved separations in zone refining may sometimes be obtained by streaming a suitable gas over the specimen. For example, Theuerer[43] has shown that boron is extremely difficult to remove from silicon by simple zone refining, but if the process is carried out in a stream of hydrogen containing small amounts of water vapour the boron can be removed preferentially from the bar. It is suggested that the process involves oxidation of both silicon and boron and evaporation of the oxidation products that condense on the walls of the tube.

Pfann[6] has described many separations attempted up to 1957, and his book should be consulted for examples, but here a few unusual separations that have been undertaken since that date will be described. Attempts have been made to separate isotopes, and the zone refining of water enriched with deuterium has been found to produce a slight concentration change. Thus, for example, when water containing 1·96 per cent. of deuterium was treated, a fraction containing 2·07 per cent. of deuterium was obtained.[44] Smith and Thomas[45] have also studied the zone refining of D_2O and H_2O mixtures, but they conclude that the practical application of the technique may be limited to attempts to prepare ultra-pure samples of D_2O or H_2O.

Peaker and Robb[46] have shown that polystyrene can be fractionated by dissolving a sample in naphthalene and placing a plug of the solid mixture at the end of a column of naphthalene previously purified by zone refining. After further passages of molten zones the column was cut up, and it was shown that some fractionation of the polystyrene according to molecular weight had taken place.

CONCLUSION

Since zone refining is a powerful purification technique and since zone levelling can produce homogeneous specimens, these methods are clearly of considerable interest to all analysts requiring reference specimens for the calibration of apparatus or for the development of analytical procedures.

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Analytical Methods Committee

ESSENTIAL OILS SUB-COMMITTEE

Application of Gas-Liquid Chromatography to Essential-oil Analysis

Interim Report on the Determination of Citronellol in Admixture with Geraniol

After Walbaum and Stephan (*Ber.*, 1900, **33**, 2307) showed that geraniol was dehydrated to a mixture of terpenes and that citronellol was converted to its formate, in almost quantitative yield, when heated with anhydrous formic acid, the method of hot formylation became widely adopted for the assay of citronellol in essential oils, such as those of rose and geranium.

It was recognised (*Schimmel's Report*, April, 1901, 50) that the method gave results slightly higher than theoretical when known mixtures of citronellol, geraniol and limonene or pinene were examined, but, in view of the obvious commercial utility of the method despite its limitations in accuracy, although not necessarily in reproducibility, it has been widely used since its inception.

Modifications in the reaction conditions have been proposed from time to time in efforts to obtain results in quantitative agreement with known percentages of citronellol. The most important of these modifications is the use of 90 per cent. formic acid in place of the 100 per cent. acid used by Walbaum and Stephan. Other modifications have involved lower ratios of acid to oil; boiling under reflux and heating in a bath of boiling water have also been proposed. All these modifications affect the ultimate result obtained for apparent citronellol content.

The reaction between citronellol and formic acid was studied by Pfau (*J. pr. Chem.*, 1921 (II), **102**, 276) and shown to be extremely complex, yielding only a minor amount of citronellyl formate, the major proportion of the esters being the mono- and diformates of citronellyl glycol (3:7-dimethyloctane-1:7-diol), accompanied by minor amounts of unchanged citronellol and terpenes and approximately 15 per cent. of polymerised residue of unknown composition. Pfau also showed that, when 100 per cent. formic acid was used, the amount of glycol diformate produced was substantially increased at the expense of citronellyl formate, compared with the results obtained by the use of 85 per cent. formic acid.

As gas - liquid chromatography is a powerful tool in the investigation of reactions of this type, the Sub-Committee considered that a preliminary study of the formylation of citronellol and geraniol in admixture would be worth while if accompanied at the same time by direct comparison of peak areas from the gas - liquid chromatograms of the mixtures thus studied.

Spectrographically pure (+)-citronellol and geraniol were used in this study, and mixtures of these constituents containing 79, 13, 37 and 57 per cent. of citronellol by weight were prepared and circulated to certain members of the Sub-Committee.

Each sample was formylated by four variants of Walbaum and Stephan's original method, and the apparent citronellol content was calculated from the ester value of the formylated oil.

Specimens of each formylated sample were hydrolysed, and the neutral ether-soluble fractions, consisting of alcohols and terpenoid hydrocarbons, were also examined by gas - liquid chromatography, except when the "recovered-alcohol" fraction was too resinous for this technique to be successfully applied.

Gas - liquid chromatography of the formylated mixtures showed the presence of three main peaks, apart from smaller peaks associated with terpenes, in all specimens. The peak associated with citronellyl formate was much larger than those derived from the glycol mono- and diesters, the last-named being by far the smallest, in contrast to Pfau's results. Polymerised material of high molecular weight would not be registered on the chromatogram.

The "recovered-alcohols" fractions all showed two peaks only, arising from citronellol and citronellyl glycol, with minor amounts of C_{10} terpenes.

The summarised results of all these determinations are shown in Table I.

TABLE I

CITRONELLOL FOUND IN VARIOUS MIXTURES BY HOT FORMYLATION AND BY GAS - LIQUID CHROMATOGRAPHY

Sample No.	Citronellol content of mixture, % w/w	Citronellol found by hot-formylation methods*				Citronellol found by gas - liquid chromatography	
		A, %	B, %	C, %	D, %	Laboratory 1, %	Laboratory 2, %
1	79	84·9	87·8	95·1	86·0	80·3 79·4 (Mean 80)	84
2	13	20·5	24·0	25·9	24·4	10·7 10·8 11·1 11·2 10·7 (Mean 11†)	12
3	37	42·5	46·3	50·0	46·8	35·9 36·5 34·9 (Mean 36)	36
4	57	63·2	67·1	71·7	65·5	58·6 57·1 (Mean 58)	60

* The hot-formylation methods used were—

A. Oil, 10 ml; 90 per cent. formic acid, 20 ml; 1 hour on sand bath.

B. Parry, "Chemistry of Essential Oils," Fourth Edition, 1922, Volume II, p. 334. Oil, 10 ml; 100 per cent. formic acid, 10 ml; 1 hour on sand bath.

C. Gildemeister and Hoffmann, "*Die Aetherische Oele*," Third Edition, 1926, Volume I, p. 732 (Walbaum and Stephan's original method). Oil, 10 ml; 100 per cent. formic acid, 20 ml; 1 hour on water bath.

D. *Synd. Nat. de Grasse, Méthode No.* 8. Oil, 10 ml; 90 per cent. formic acid, 20 ml; 1 hour on water bath, with shaking during first 5 minutes.

† This discrepancy of 11 against 13 was probably due to non-linearity of the detector in the instrument used.

It will be observed that method A in Table I gives results nearest to the theoretical, but even so the divergencies from the latter are considerable.

CONCLUSIONS

The Sub-Committee considers that any reference method should be free from demonstrable inherent errors and, in consequence, does not support any method of hot formylation for the determination of citronellol in essential oils. The Sub-Committee is further of the opinion that the application of gas - liquid chromatography to the determination of citronellol in essential oils is worth further study.

The Determination of Organo-mercury Residues in Plant Material*

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Procedures proposed for the determination of micro amounts of mercury in biological materials are reviewed.

THE determination of organo-mercury residues, in common with those of other pesticide chemicals used on harvested crops, has become very important in view of the concern now shown in the amount of residue likely to be present at the time of consumption. It is important that such residues, if present, should not constitute a hazard to the consumer.

Only a limited number of analytical papers dealing with the determination of organo-mercury residues in plant material have been published, and there is consequently little information on suitable alternative analytical techniques. The majority of the published information is reported in terms of mercury determined after destruction of all organic matter in the sample under test. The toxicologist has, therefore, little information as to whether the organo-mercury compound remains intact on the plant surface, or is broken down to other compounds by weathering or by natural physiological processes within the plant cells.

Martin and Pickard[1] have found that phenylmercuric nitrate or chloride remains unchanged in chemical form after exposure on apples for a limited period towards the end of the season. These workers have also shown that the mercury content of a solvent wash of the macerated fruit gave lower results than did a treatment involving total destruction of the organic matter by a wet-oxidation procedure. It is probably for this reason that most workers have chosen an analytical method involving a total or partial destruction of organic matter before the determination of the ionic mercury produced.

Arthington and Hulme[2] have described a technique for determining mercury on the skins of apples. These workers freeze-dry the sample before analysis to reduce the dilution effect in the subsequent wet acid-digestion process and determine the mercury in the digested solution by the reversion technique of Irving, Andrew and Risdon.[3] Ford and Burkholder[4] dissolve surface residues on apples by boiling them in 3 per cent. nitric acid and determine the mercury colorimetrically with di-β-naphthylthiocarbazone. Beidas and Higgons[5] use the wet-oxidation method of Klein[6] and the dithizone extractive titration technique to determine residues on tomatoes.

The Department of the Government Chemist has recently issued an interim report[7] on the estimation of mercury in apples. With a few minor modifications the method described is that of Abbott and Johnson,[8] who found that the Klein method[6] of digestion, although suitable for tomatoes, gave low recoveries of mercury on apples. They recommend the combustion method of Laug and Nelson[9] as modified by Kunze,[10] that is, combustion in a mixture of sulphuric and nitric acids (3 + 1) with metallic selenium. The mercury is extracted from the treated acid digest by the method of Klein.[6] By using this method, with 5 μg of mercury added as phenylmercuric acetate to the digestion mixture, with and without 50 g of macerated apple pulp, we have obtained recoveries ranging from 4·4 to 4·9 μg of mercury in seven determinations; one other determination gave 3·9 μg. The residues found, again with this method, on apples after treatment with "PP" Liquid Mercury Plus in commercial orchards are shown in Table I. Each determination was carried out by taking ten apples at random from the sample, quartering them and using opposite quarters chopped and well mixed. A 50-g aliquot was taken for analysis.

The wet-digestion methods available are extremely time-consuming and require the skill of an experienced operator. There is a very considerable number of papers in the literature giving a very wide range of methods for the determination of micro amounts of mercury in urine and animal tissues, and certain techniques are available on which there is apparently no published information as to their suitability for plant materials.

* Presented at the meeting of the Society on Wednesday, February 4th, 1959.

Gorsuch[11] has studied losses of mercury occurring during various wet-oxidation procedures, using irradiated mercury and gamma counting the fractions from a modified digestion apparatus. In the presence of organic matter, considerable losses of mercury were shown to occur unless special precautions were taken. He comments that methods involving the distillation of mercury from the sample, rather than trying to retain it in the digestion stage, would seem to offer considerable scope for development. This would certainly appear to

TABLE I

MERCURY RESIDUES ON APPLES AT HARVEST TREATED WITH "PP" LIQUID MERCURY PLUS

Variety	Rate of application	Number of applications	Date of last application	Period between last application and sampling, weeks	Residues found in whole fruit, as mercury, p.p.m.
Cox	200 gall. per acre (mercury, 30 p.p.m.)	8	July 10th	10	0·08, 0·07, 0·07
	200 gall. per acre (mercury, 20 p.p.m.)	8	July 10th	10	0·03, 0·04, 0·04
	Nil	Nil	—	—	0·02, 0·02, 0·02
Worcester Pearmain*	50 gall. per acre (mercury, 100 p.p.m.)	6	June 30th	13	0·11, 0·12, 0·12
Derby* ..		6	June 30th	13	0·06, 0·07
Laxton* ..		6	June 30th	13	0·07, 0·08
Bramley A*		6	June 30th	13	0·07, 0·05
Bramley B*		5	about July 5th	17	0·03, 0·05, 0·05

* Untreated samples not available.

be worth investigating with plant materials. The use of 50 per cent. hydrogen peroxide as a digesting agent at low temperatures as described by Polley and Miller[12] would also be worth further investigation.

For the extraction stage, dithizone has been used almost universally, and it would appear that the use of di-β-naphthylthiocarbazone does not offer any advantage now that the photochemical decomposition of mercuric dithizone can be prevented by the addition of acetic acid. Dithizone, however, is not specific for mercury, and consequently, in its application to residue work, the extraction of copper, particularly, must be guarded against. Further, the sensitivity of the reagent to oxidation necessitates complete removal of all oxidising agents used in the digestion. Irving *et al.*[3] have shown that quantitative determination of mercury in the presence of copper is best effected by extraction with a solution of dithizone in chloroform from a sulphuric acid solution adjusted to a pH of about 1 and as free from halide ions as possible.

All the available absorptiometric methods for determining the mercury complex of dithizone fall within one of three distinct groups: the mixed-colour method, the monocolour method and the Irving reversion technique.[3] It has been found in these laboratories that when applying the mixed-colour method recommended by Abbott and Johnson,[8] great care has to be taken at all stages of the extraction to prevent the dithizone becoming oxidised, since this will affect the blank values measured at 490 mμ. It would appear that the alkaline stripping method as recommended by the Joint Committee of the Society for Analytical Chemistry and the Association of British Chemical Manufacturers,[13] or the use of dilute ammonia as described by Milton and Hoskins,[14] would greatly reduce this difficulty. A further modification of this procedure has been described by Isaacs, Morries and Stuckey,[15] in which excess of dithizone is removed by passing the solution down an alumina column. These workers have shown that copper is also removed by this procedure, and it would appear to be a particularly valuable technique, since the excess of dithizone and the copper can be removed in one operation.

The photometric method of Ballard and Thornton,[16] adapted to air samples and biological materials by Monkman, Maffett and Doherty,[17] would also appear to be a promising approach for plant materials after a digestion stage. The mercury is collected on a pad of cadmium sulphide and, after being washed and dried, is volatilised in a micro gas chamber heated to 450° to 550° C. The concentration of vapour is determined by a detector cell containing a photo-electric cell and monochromatic light source. The output of the photo-electric cell is plotted on a chart recorder and the area under the curve traced is measured and compared with

areas obtained for known amounts of mercury. It is claimed that this method provides for the determination of 0·1 to 10 ± 0·1 μg of mercury, and greater sensitivity can be obtained by suitable modification of the recorder. The authors state that an experienced operator can carry out 20 to 25 determinations per day.

From this summary it would appear that there is ample scope for developing alternative and probably much more rapid methods of analysis for small amounts of organo-mercury compounds in plant materials, but no one method, or combination of methods, can be recommended without experimental results.

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A Colour Reaction for Detecting and Determining Chromones and Related Compounds

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Certain chromones react with alkaline *m*-dinitrobenzene to form an intense blue or purple colour; this reaction is of value for their detection and quantitative determination. Structural prerequisites for colour formation have been investigated. More than 100 compounds were tested, and from these tests it can be concluded that simple chromones having a methyl or methylene substituent in the 2-position form a characteristic blue or purple colour. Hydroxyl substituents interfere with colour formation; methoxy, methyl and halogen substituents do not. 3-Methylchromone produces no colour. A typical quantitative application of the reaction is described.

During a study of the metabolism of 2:5:8-trimethylchromone, a synthetic coronary vasodilator, it was found that certain chromone derivatives formed a stable blue to purple colour when treated with ethanolic alkali in the presence of *m*-dinitrobenzene.[1,2] The reaction was found to be applicable to the detection and quantitative determination of these chromones in blood, urine and various animal tissues. In order to determine the structural unit responsible for the specificity of the colour reaction, more than 100 chromones and related compounds were subjected to the conditions of the test. This reaction was first utilised by Zimmermann[3] for determining certain sex hormones and has also been used in the detection and determination of cardiac glycosides.[4,5]

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The compounds tested were kindly presented from various sources (see p. 698). They were used as received, with no further purification.

Colorimetric measurements were made in matched 1·00-cm Corex cells with a Beckman DU spectrophotometer.

Method

Reagents—

m-*Dinitrobenzene solution*—Dissolve 2 g of *m*-dinitrobenzene in 100 ml of 95 per cent. ethanol.

Potassium hydroxide solution, ethanolic—Dissolve 12 g of analytical-reagent grade potassium hydroxide in 100 ml of 95 per cent. ethanol, and filter. Prepare this solution daily.

Ethanol - water solvent—Mix equal volumes of 95 per cent. ethanol and distilled water.

Qualitative procedure—

To a few crystals or a small drop of the compound to be tested were added 0·3 ml of *m*-dinitrobenzene solution and then 0·6 ml of ethanolic potassium hydroxide solution. Colour was allowed to develop for about 30 seconds, after which the mixture was diluted with 5 ml of 95 per cent. ethanol. The dilution slows or stops the reaction and usually stabilises the colour.

Quantitative procedure—

2:5:8-Trimethylchromone was determined as described below. A sample was accurately weighed and dissolved in chloroform. Aliquots of the solution containing from 5 to 100 μg of the chromone were placed in test-tubes and evaporated to dryness by means of a current of air. In each test-tube was placed a 0·5-ml portion of *m*-dinitrobenzene solution, the test-tubes were swirled and then 1·0-ml portions of ethanolic potassium hydroxide solution were added to the contents of each test-tube, which were again swirled. Maximum colours were attained in 5 minutes; at the end of this time, 4·0-ml portions of ethanol - water solvent were placed in each test-tube.

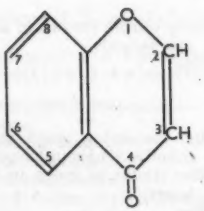
Results

Qualitative—

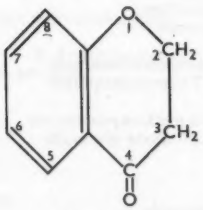
Table I shows a list of the compounds tested and the colours observed. Unless otherwise stated, the blue or purple colours are intense and essentially stable for at least 1 hour. As only the blue or purple colours are intense, only these colours or colours close to them are tabulated and considered as a positive reaction. Failure either to give the aforementioned colour reactions or to form a colour at all is indicated by a dash.

Several of the chromones and related compounds tested did not give a typical reaction, in that the colours produced were neither blue nor purple; sometimes the colours were also faint. Some other similar compounds gave completely negative reactions.

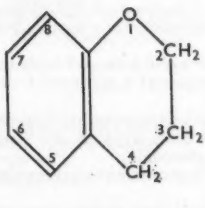
To clarify the type of structure necessary to produce a typical colour, some intermediates and related compounds were similarly tested. The results are also shown in Table I; again, the blue or purple colours produced are intense unless otherwise stated.



Chromone



Chromanone



Chroman

TABLE I

COLOURS PRODUCED BY VARIOUS COMPOUNDS

Compound	Colour formed	Source
2-Methylchromones—		
2-Methylchromone	Purple	5
2:5:8-Trimethylchromone	Purple	1, 5
2:5:8-Trimethylchromone bromide	Purple	1
2:3:5:8-Tetramethylchromone	Purple	1
2:6-Dimethylchromone	Purple	2
6-Chloro-2-methylchromone	Blue	2
6-Chloro-2:5:7-trimethylchromone	Purple	1
3-Hydroxy-2-methylchromone	—	5, 10
7-Hydroxy-2-methylchromone	Rose-purple (fades in 2 hours)	5
7-Methoxy-2-methylchromone	Purple	2
6-Methoxy-2-methylchromone	Bluish purple	2
5:8-Dimethoxy-2-methylchromone	Purple	10
7:8-Dimethoxy-2-methylchromone	Blue	2
6:7-Dimethoxy-2-methylchromone	Purple	10
2-Methyl-5:6:7-trihydroxychromone	—	3
5:7-Dihydroxy-3-methoxy-2-methylchromone	—	9
6:7-Dihydroxy-5-methoxy-2-methylchromone	—	3
5:7-Dimethoxy-7-hydroxy-2-methylchromone	Purple	3
2-Methyl-5:6:7-trimethoxychromone	Purple	3
2-Methyl-5:7:8-trimethoxychromone	Purple	2
3-Methoxy-2-methyl-5:7:8-trihydroxychromone	—	9
2-Methyl-3:5:7:8-tetramethoxychromone	Purple	9
5:7-Dihydroxy-6-formyl-2-methylchromone	—	3
6-Formyl-7-hydroxy-5-methoxy-2-methylchromone	Purple (purplish blue on dilution with ethanol)	3
6-Formyl-7-hydroxy-5-methoxy-2-methylchromone anil	Purple (purplish blue on dilution with ethanol)	3
6-Formyl-7-hydroxy-5-methoxy-2-methylchromone oxime	Purple (purplish blue on dilution with ethanol) Fades in 1 hour	3
8-Bromo-6-formyl-7-hydroxy-5-methoxy-2-methylchromone	Purple	3
5:7-Dihydroxy-6-formyl-2-methyl-8-nitrochromone	—	3
6-Formyl-7-hydroxy-5-methoxy-2-methyl-8-nitrochromone	Purple (purplish blue on dilution with ethanol)	3
8-Methoxy-2-methyl-6:7-benzochromone	Blue	7
5-Methoxy-2-methyl-4′:5′:6:7-furanochromone (visnagin)	Purplish blue	3
8-Bromo-5-methoxy-2-methyl-4′:5′:6:7-furanochromone	Bluish purple (purple precipitate)	3
5:8-Dihydroxy-2-methyl-4′:5′:6:7-furanochromone	—	3
8-Amino-5-hydroxy-2-methyl-4′:5′:6:7-furanochromone	Purplish black (fades to red)	3
5:8-Dimethoxy-2-methyl-4′:5′:6:7-furanochromone (khellin)	Blue	3
5-Dimethylamino-8-methoxy-2-methyl-4′:5′:6:7-furanochromone	Blue	3
3-Acetyl-2-methylchromone	Rose-purple	5
3-Benzoyl-2-methylchromone	—	5
3-Benzyl-2-methylchromone	Blue-purple	5
Chromones having 2-substituents other than methyl—		
2-Chromonealdehyde	—	5
2-Chromonecarboxylic acid	—	1, 10
*iso*Propyl-2-chromonecarboxylate	—	5
5:8-Dimethyl-2-chromonecarboxylic acid	—	1
Butyl-5:8-dimethoxy-2-chromonecarboxylate	—	10
Butyl-6:7-dimethoxy-2-chromonecarboxylate	—	10
2-Morpholinomethylchromone hydrochloride	Dirty purple (brown in a few seconds)	5
2-Chloromethyl-6-formyl-7-hydroxy-5-methoxychromone	Blue	3
2-Hydroxymethyl-5-methoxy-4′:5′:6:7-furanochromone (khellol)	—	3
6-Formyl-2-hydroxymethyl-7-hydroxy-5-methoxychromone	—	3
2-Methyl-5-methoxy-4′:5′:6:7-furanochromone glucoside (khellol glucoside)	Blue becoming green in 3 minutes (fades in 1 hour)	3
2-(β-Dimethylaminoethyl)chromone	Blue (fades to green in 2 hours)	5
2-(β-Morpholinoethyl)chromone hydrochloride	Blue	5
2-(3′:4′-Methylenedioxybenzoyl)chromone	—	5

TABLE I—*continued*

Compound	Colour formed	Source
2-Carboxyethyl-5:8-dimethoxy-4′:5′:6:7-furanochromone	—	3
5:8-Dimethyl-2-(4′-methoxystyryl)chromone	—	1
5:8-Dimethyl-2-(3′:4′-methylenedioxystyryl)chromone	—	
2-Styrylvisnagin	—	3
2-Styryl-8-methoxy-6:7-benzochromone	—	7
2-Styrylkhellin	—	3
2-Phenylchromone (flavone)	—	11
2-(4′-Pyridyl)chromone	—	5
3:3′:4′:5:7-Pentahydroxy-2-phenylchromone (quercitin)	—	11
2-(4′-Tetrahydropyranyl)chromone	—	5
4-Thio-2-chromonecarboxylic acid	—	10
Chromones having no 2-substituents—		
Chromone	—	1
3-Methylchromone	—	4, 5
3-(Dimethylaminomethyl)-7-methoxychromone	—	2
6-Hydroxy-3-ethylchromone	—	12
6-Hydroxy-3-propylchromone	—	12
6-Hydroxy-3-butylchromone	—	12
6-Hydroxychromone	—	12
7-Hydroxychromone	—	12
5-Methoxychromone	Rose-red (fades in ethanol)	12
6-Methoxychromone	Rose (fades rapidly to orange)	2
7-Methoxychromone	Rose (fades rapidly to orange)	2, 12
7-Hydroxy-8-nitrochromone	—	12
7-Methoxy-8-nitrochromone	—	12
5-Methoxy-8-nitrochromone	—	12
7-Methoxy-8-aminochromone	—	12
6-Hydroxy*iso*flavone	—	11
7:8-Dihydroxy*iso*flavone	—	11
5:6:7-Trimethoxy*iso*flavone	—	11
Chromans—		
2:2-Dimethylchroman	Pale purple	8
2:2-Dimethyl-5-hydroxychroman	—	8
2:2-Dimethyl-7-hydroxychroman	Pale purple	8
2:2-Dimethyl-5-methoxychroman	—	8
2:2-Dimethyl-7-methoxychroman	—	8
2:2-Dimethyl-8-methoxychroman	Pale purple	8
2:2-Dimethyl-8-chromancarboxylic acid	—	8
Chromanones—		
6-Methoxy-4-chromanone	Purple (perceptible fading in 1 hour)	6
7-Methoxy-4-chromanone	Purple (perceptible fading in 1 hour)	6
8-Methoxy-4-chromanone	Purple (perceptible fading in 1 hour)	6
5:7-Dimethoxy-4-chromanone	Purple (fades in a few minutes)	6
5:8-Dimethoxy-4-chromanone	Rose-purple (fades in a few minutes)	6
6:7-Dimethoxy-4-chromanone	Bluish purple (fades in ~45 minutes)	6
6:8-Dimethoxy-4-chromanone	Purple (fades in ~45 minutes)	6
7:8-Dimethoxy-4-chromanone	Purple (fades in ~45 minutes)	6
7-Carbomethoxy-2:2-dimethyl-6-hydroxy-4-chromanone	Pale purple (faint green on dilution with ethanol)	8
6-Carbomethoxy-2:3:8:9-tetrahydro-2:2:8:8-tetramethyl-4H, 10H-benzo(1:2-*b*, 3:4-*b*′)dipyran-4:10-dione	—	8
Intermediate and related compounds—		
Acetophenone	Purple (rapidly becomes red)	—
o-Hydroxyacetophenone	Red (rapidly fades)	—
p-Hydroxyacetophenone	Pale rose	—
p-Methoxyacetophenone	Purplish red	—
2-Hydroxy-3:6-dimethylacetophenone	—	1
α-Bromo-*p*-nitroacetophenone	Rose-red	—
Ethyl *o*-hydroxybenzoylpyruvate	—	1
Crotonic acid	—	—
Ethyl β-anilinocrotonate	Purple (changes to brown in a few seconds)	1
Ethyl β-chlorocrotonate	Purple (changes to brown in a few seconds)	1

TABLE I—*continued*

Compound	Colour formed	Source
β-(2:5-Dimethylphenoxy)crotonic acid	—	1
Phenol	—	—
Hydroquinone	—	—
Resorcinol	Pale purple (rapidly fades)	—
Phloroglucinol	—	—
Benzoquinone	—	—
Anthrone	—	—
Diethyl chlorofumarate	—	1
trans-Cinnamic acid	—	—
Ethyl cinnamate	Purplish red (faint)	—
Ethyl salicylate	—	—
Valeraldehyde	—	—
Caprylaldehyde	Purplish red	—
2-Ethylhexaldehyde	Purplish red (fades on dilution with ethanol)	—
Cinnamaldehyde	Red-brown	—
Xanthone 1:3-Dihydroxyxanthone Morellin Tetrahydromorellin Artocarpin Tetrahydroartocarpin α-Toxicarol	—	11
Toxicarol	—	8
1-(*p*-Thiophenyl)-3-methyl-5-pyrazolone (technical)	Red	—

SOURCES

1. Dr. N. H. Leake and M. Fielden, S.E. Massengill Co., Bristol, Tennessee, U.S.A.
2. Dr. P. F. Wiley, Eli Lilly Research Laboratories, Indianapolis, Indiana, U.S.A.
3. The Memphis Chemical Co., Cairo, Egypt (Dr. A. Schönberg, University of Fuad I)
4. Dr. C. Mentzer, University of Lyon, France
5. Dr. J. Schmutz, Dr. A. Wander S.A., Berne, Switzerland
6. Dr. G. D. Thorn, Science Service Laboratory, Department of Agriculture, London, Ontario, Canada
7. Dr. S. Wawzonek, State University of Iowa, Iowa, U.S.A.
8. Dr. R. Huls, University of Liège, Belgium
9. Dr. D. Donnelly, University College, Dublin, Eire
10. Dr. L. Vargha, Pharmaceutical Research Institute, Budapest, Hungary
11. Dr. K. Venkataraman, University of Bombay, India
12. Prin. B. V. Bhide, Sir Parashurambhau College, Poona, India

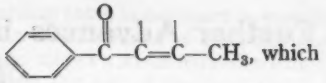
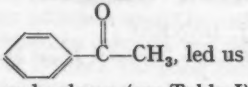
QUANTITATIVE—

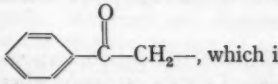
The results with 2:5:8-trimethylchromone are apparently typical of those that may be expected for other appropriate 2-methylchromones.

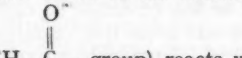
The final colour, which was stable for several hours, was measured against a reagent blank solution at 520 mμ. The absorption spectrum for 2:5:8-trimethylchromone had a single maximum at 520 mμ ($E^{1\%}_{1\,cm} = 650$) in the range 220 to 1000 mμ. A calibration graph at 520 mμ was linear over the range 5 to 100 μg of 2:5:8-trimethylchromone per sample. Identical optical-density readings were obtained for a 50-μg sample both 7 minutes and 1 hour after the procedure described had been completed.

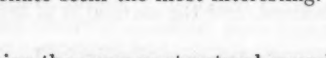
DISCUSSION OF RESULTS

Our observations indicate that, for a chromone to produce the characteristic blue to purple colour, it must be substituted with a methyl or methylene group in the 2-position. In addition, hydroxyl groups must be absent from the 2- or 3-position and no more than one hydroxyl group may be present anywhere in the benzene ring. Other groups, such as halogen, alkyl, alkoxyl, nitro and formyl, may be present in the benzene ring and would not be expected to interfere with colour production. The colours produced by some of these compounds are not stable, but they mostly last long enough to be of quantitative use. It is interesting to note that there is only a narrow range of qualitative difference between the colours produced by variously substituted 2-methylchromones.

The fact that 2-methylchromones contain the grouping  , which resembles acetophenone,  , led us to test various acetophenones. In general they produced purple to red colours (see Table I).

Chromanones were also found to produce stable purple colours, as expected, since they contain the grouping  , which is present in acetophenone. The chromans have no grouping analogous to that in 2-methylchromone, acetophenone or chromanone and were not therefore expected to produce a colour. The extremely slight colours formed by three of the chromans tested are assumed to have been produced by traces of impurities, since the other four similar chromans produced no colour.

The two 3-methylchromones tested did not produce colours, nor were they expected to do so, since they contained no grouping comparable to that of the compounds giving a positive reaction. Since the colour-producing compounds react with benzaldehyde to form benzylidene derivatives and those producing no colour do not,[6] then either the group or the group must be involved in the colour reaction. Carr[7] has applied the colour produced by alkaline sodium 3:5-dinitrobenzoate and creatinine to the determination of the latter substance. He postulated[8] the formation of a coloured addition complex when the electronegative centre of the resonance hybrid anion of creatinine (and other substances having a  group) reacts with the resonance hybrid dipole of sodium 3:5-dinitrobenzoate. The colour reaction described in this paper can be explained in a similar way.

Several miscellaneous compounds were tested. Of these, ethyl β-anilinocrotonate and ethyl β-chlorocrotonate seem the most interesting. Both are structurally analogous to 2-methylchromone, having the common structural grouping  , and produce a transient purple colour. The colours produced by acids are generally less stable than those from the corresponding esters. Hence, the related β-(2:5-dimethylphenoxy)crotonic acid and crotonic acid do not form colours. Esters of both these acids would be expected to produce a blue or purple colour, but were not available for testing.

Conclusions

Simple chromones with a methyl or methylene substituent in the 2-position form a characteristic and intense blue or purple colour with alkaline *m*-dinitrobenzene. Hydroxyl substituents interfere with this reaction, but methoxy, methyl and halogen substituents do not. The reaction appears to be suitable for quantitative adaptation.

References

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3. Zimmerman, W., *Z. physiol. Chem.*, 1936, **245**, 47.
4. Raymond, W. D., *Analyst*, 1938, **63**, 478.
5. Hassall, C. H., and Lippman, A. E., *Ibid.*, 1953, **78**, 126.
6. Heilbron, I. M., Barnes, H., and Morton, R. S., *J. Chem. Soc.*, 1923, **123**, 2559.
7. Carr, J. J., *Anal. Chem.*, 1953, **25**, 1859.
8. ——, Ph.D. Thesis, New York University, 1952.

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Further Advances in the Absorptiometric Determination of Dissolved Oxygen

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Further work is described on the determination of dissolved oxygen with 3:3′-dimethylnaphthidine by a simpler and more sensitive procedure than that originally proposed. A precision of ±0·0026 p.p.m. has been achieved, and possible interference is indicated.

A METHOD has been described[1] for the absorptiometric determination of dissolved oxygen in boiler-feed water; it depends on the intensity of colour produced when a solution of 3:3′-dimethylnaphthidine is added to the solution of iodine formed by the Winkler reaction. The use of 3:3′-dimethylnaphthidine has been proposed by Belcher, Nutten and Stephen for determining chlorine in water.[2]

The method for oxygen was simple and accurate, but could be criticised on the grounds that—

(*i*) The calibration graph was prepared indirectly from standard iodine solutions.
(*ii*) No evidence was then available that the Winkler method was stoicheiometric at oxygen contents below 0·01 p.p.m.
(*iii*) The boiler-feed water available for field tests of the method always contained more than 0·01 p.p.m. of oxygen.
(*iv*) The pH at the end of the Winkler reaction was such as to inhibit the oxidation of 3:3′-dimethylnaphthidine, and a buffer solution had to be added before the reagent.

This investigation was designed to produce a method free from these faults. The increase in boiler-operating pressures to above 2000 lb per sq. inch has necessitated procedures capable of determining 0·007 p.p.m. or less of dissolved oxygen with a precision of ±0·002 p.p.m., and any proposed method should meet this requirement.

Potter[3,4] and Potter and White[5,6,7] have exhaustively studied the determination of dissolved oxygen and have proved the validity of Winkler's method[6] at oxygen concentrations below 0·001 p.p.m. They have proposed a precise and accurate method[7] involving specially designed sampling and processing tubes[4] and an electrometric titration for determining the liberated iodine. Considerable experience and manipulative skill are necessary before reproducible results can be obtained, and the method is of limited value as a routine procedure.

Stones[8] has applied a modification of the American Society for Testing Materials' difference method[9] and includes the determination of iodine with dimethylnaphthidine, as proposed by Banks.[1] In the difference method, two portions of water having equal volumes are collected. With one (the sample), the normal Winkler procedure is carried out, but with the other (the blank), the reagents are added in reverse order to prevent reaction of the dissolved oxygen with manganous hydroxide, manganous ions being added last to the already acid solution. The difference in the amounts of iodine liberated is a measure of the oxygen present, any oxidising impurity having been allowed for. In the A.S.T.M. method, an oxidant, usually iodine, is added to the potassium hydroxide - potassium iodide solution to compensate for any reducing interference, the same amount being added to sample and blank. Potter and White[7] used potassium iodate for the same purpose. In the A.S.T.M. procedure, the oxidant in the sample, added with the alkaline iodide reagent before any other processing reagent, arrives at a high pH; in the blank, however, the alkaline iodide *plus* oxidant are dispensed into an acid medium. This may not cause any difference in behaviour towards impurities, but was thought to be a disadvantage. In the proposed procedure, iodine solution is added to both sample and blank as a final separate reagent, both solutions being acid at the time.

One of the outstanding features of Potter and White's technique is the small volume of reagents used. Only 0·125 ml of each is added, as compared with 1 or 2 ml recommended

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in most modifications of Winkler's procedure. Potter's sampling tube[4] is designed to permit this small amount to be dispensed accurately. The use of a small volume of concentrated reagents renders their preliminary de-aeration unnecessary and allows a small fixed correction to be applied for oxygen in reagents.[7] It was expected that the pH of the final treated sample after these reagent additions would be sufficiently high to render buffering unnecessary before the dimethylnaphthidine solution was added.

The disadvantages of Potter's sampling tube are the high degree of manipulative skill required and its cost in relation to probable breakage in routine use. An alternative method of sampling that permits equal accuracy in reagent addition has been proposed by Holland.[10] The samples are collected by the submerged-bottle technique and the bottles are sealed with Suba-seal stoppers. Reagents are dispensed from a graduated hypodermic syringe through the centre of the stopper, ingress of air being prevented by a seal of water lying in the cup formed by the upturned edges of the stopper and by the slight positive pressure induced in the sample bottle by the addition of even 0·1 ml of reagent solution. One such stopper can accommodate from twelve to twenty insertions of a No. 20 hypodermic needle before it is discarded. It was proposed to use this method of sampling, which also had the advantage that all reagent additions, including that of the dimethylnaphthidine, could be carried out in the same vessel.

Experimental

In the early experimental work, the colour of the oxidised dimethylnaphthidine faded seriously under the original conditions of colour development.[1] This was traced to the solid reagent, and experience has shown that, if the solid is allowed to become damp, slow oxidation occurs and causes impaired colour intensity and stability. The reagent is best stored in an ampoule placed inside a tightly stoppered boiling-tube containing silica gel. It is now also known that the reagent solution need not be set aside before use[1] and is, in fact, best prepared freshly at frequent intervals, *e.g.*, weekly.

The effect of the pH of the treated sample from Potter and White's procedure was first investigated. Solutions were prepared from 0·001 *N* iodine[1] to cover the range 0·0 to 0·08 p.p.m. of dissolved oxygen. The dilution medium was prepared by adding, per litre of distilled water, 0·5 ml each of diluted sulphuric acid (3 + 1) and a solution containing 700 g of potassium hydroxide and 150 g of potassium iodide per litre. Manganous sulphate solution was not added, since it was only desired to establish correct conditions of acidity. A solution of 3:3'-dimethylnaphthidine in glacial acetic acid was added to each of the standard solutions, and the extinction of each resulting coloured oxidation product was measured in 4-cm cells with a Spekker absorptiometer, Ilford No. 605 filters being used. The graph of extinction against concentration of dissolved oxygen was linear and passed through the origin, and intensity and stability of colour were better than when a buffer was used.

This experiment was repeated with standards containing dissolved oxygen in place of iodine. Potter and White[6] de-aerated water by means of nitrogen in an elaborate apparatus and then added known volumes of air-saturated water to provide solutions of known oxygen content. I used a simpler apparatus to provide the same result. A flat-bottomed 1000-ml flask was fitted with a rubber stopper through which passed an inlet tube drawn out to a coarse jet and reaching almost to the bottom of the flask and a short outlet tube. The stopper had a hole through which reagents could be added from a microburette fitted with a long extension jet fixed by polythene tubing and capable of dipping under the surface of the water; this hole was closed during de-aeration by means of a small rubber stopper.

About 900 ml of distilled water were heated to boiling-point in the flask and a rapid stream of nitrogen was passed through the inlet tube for 10 to 15 minutes. A 0·4-ml portion of manganous sulphate solution was added before boiling to ensure its de-aeration also. The flask was cooled under running water, the flow of nitrogen being maintained, and the inlet tube was then raised above the surface of the water to provide a nitrogen seal. A 0·4-ml portion of de-aerated alkaline iodide solution was added from a microburette through the hole in the stopper, the outlet tube being temporarily blocked with a finger to ensure positive nitrogen pressure around the burette. The flask was gently swirled to disperse the precipitated manganous hydroxide, a measured volume of air-saturated water was added from a microburette and the flask was again gently swirled to mix its contents. Finally, 0·4 ml of diluted sulphuric acid (3 + 1) was added, the nitrogen supply was stopped and the stopper was removed. The total volume of treated water was measured, dimethylnaphthidine

solution was added to 100 ml of it and the colour intensity was measured as before. The amount of added oxygen was found from the values given by Truesdale, Downing and Lowden.[11] The graph of oxygen present against extinction was linear over the range 0·0 to 0·06 p.p.m. and passed through the origin. Blank tests on the de-aerated water yielded no colour with dimethylnaphthidine.

This method was open to criticism, as the oxygen was added after precipitation of the manganous hydroxide. It was feared that if the air-saturated water were added first, oxygen might be transferred to the nitrogen-rich atmosphere in the flask. It was found that a similar apparatus could be used to fill with de-aerated water a sampling tube of the type described by Potter. A flat-bottomed 1500-ml flask was provided with a rubber stopper fitted with long inlet and short outlet tubes only. The outlet was attached to the inlet of the sampling tube, which was filled with distilled water and supported in a clamp, outlet end down. When the nitrogen-scrubbed water was boiling, the taps of the sampling tube were turned so that the water was expelled via the outlet and the tube was scrubbed by the nitrogen - steam mixture. The flow of nitrogen was maintained during cooling. The sampling tube was then clamped in the sampling position, *i.e.*, outlet end up, and the flask was inverted so that the nitrogen forced water through the outlet and into the sampling tube. Water was allowed to overflow from the sampling tube for as long as possible before the taps were closed.

A short piece from a broken microburette was attached to the inlet of the sampling tube by polythene tubing, and air-saturated water was added from it, the taps of the sampling tube being used to control the flow from the burette. Reagents were added in the normal manner,[4] dimethylnaphthidine solution was added to each treated sample and the colour intensities were measured. The graph of colour intensity against oxygen present coincided with that obtained from the previous series of tests and was also identical with lines obtained when solutions of iodine were substituted for dissolved oxygen. A calibration graph can therefore readily be prepared from a simple standard without the necessity to de-aerate water and add oxygen.

It was attempted to prove the validity of the submerged-bottle and Suba-seal technique in a manner similar to that already described, but narrow-necked 250-ml reagent bottles could not be filled with de-aerated water without ingress of air, possibly because of the limited rate of flow available. De-aerated boiler-feed water was therefore used to investigate the sampling technique, and supplies of water often containing less than 0·01 p.p.m. of oxygen were available. Further, since it was intended to add sufficient iodine to ensure that the equivalent of about 0·01 p.p.m. of oxygen remained in the blank after treatment, the concentration of oxygen originally in the sample was of less importance than the accurate recovery of added oxygen.

For adding reagents by syringe, standard 20-gauge stainless-steel needles were at first used, but severe corrosion occurred with diluted sulphuric acid (3 + 1). Platinum - iridium needles were obtained for use with this reagent, but stainless-steel needles were satisfactory for all other reagent solutions. Air-saturated water and oxidant were added from a syringe controlled by a micrometer. The latter was of simple construction, made in the Station workshop and accurately calibrated.

Method

Apparatus—

Sample bottles—Borosilicate-glass narrow-necked 250-ml bottles.

Suba-seal stoppers—Stoppers having a plug 19 mm in diameter were the most convenient.

Hypodermic syringes—Use an all-glass Record 1-ml syringe, graduated in 0·1-ml divisions, for each reagent. Each syringe should be fitted with a No. 20 needle, 0·5 inch long (these needles are approximately 0·45 mm in diameter). Use a platinum - iridium needle for the diluted sulphuric acid and stainless-steel needles for the other reagents. The syringe for the iodine solution should be fitted with a calibrated micrometer.

Reagents—

All materials should be of recognised analytical-reagent grade.

Manganous sulphate solution—Dissolve 400 g of manganous sulphate tetrahydrate in water, and dilute to 1 litre.

Alkaline iodide solution—Dissolve 700 g of potassium hydroxide and 150 g of potassium iodide in water, cool, and dilute to 1000 ml.

Sulphuric acid, diluted (3 + 1).

Iodine solution, approximately 0·005 N.

3:3′-*Dimethylnaphthidine solution*—Dissolve 0·025 g of 3:3′-dimethylnaphthidine in 50 ml of glacial acetic acid; warm to assist solution. Cool before use.

PROCEDURE—

Attach rubber tubing to the sampling point, and lead through a Y-junction to the bottoms of two sample bottles standing in a container sufficiently tall to give at least 3 inches submergence. Lodge two Suba-seal stoppers between the bottles and the wall of the container, so that they will be immersed in water. Maintain a brisk flow of water, efficiently cooled if necessary, and allow at least ten sample volumes to flow to waste. Remove the sample leads, ensure that all air has been expelled from the hollow plugs in the stoppers and from the serrations around them, and insert the stoppers in the necks of the bottles. Check for complete absence of air bubbles. Leave each stopper with its soft lip upturned to form a small cup.

To dispense reagents, place a little distilled water in the cup to form a seal, and fill a hypodermic syringe with the appropriate solution (expel air from the needle by gentle pressure on the piston). Pass the needle through the centre of the stopper, and add 0·1 ml of solution from the syringe. Maintain a slight pressure on the piston when withdrawing the needle, in order to prevent liquid passing from bottle to syringe. Wash the cup with distilled water, invert the bottle to mix, and place water in the cup ready for the next addition. Add 0·1-ml portions of the reagents in the order shown below—

Sample	Blank
Alkaline iodide solution	Alkaline iodide solution
Manganous sulphate solution	Diluted sulphuric acid (3 + 1)
Diluted sulphuric acid (3 + 1)	Manganous sulphate solution
Iodine solution	Iodine solution

Use the platinum - iridium needle for adding the diluted sulphuric acid and the micrometer-controlled syringe for adding the iodine solution. Thorough mixing is essential at each stage and is especially important after the addition of acid. All the precipitate in the bottle containing the sample must be dissolved before iodine solution is added.

Remove the Suba-seal stopper from each bottle, reject a little of the contents, add 1 ml of 3:3′-dimethylnaphthidine solution, and measure the extinction of the sample against the blank in 4-cm cells with a Spekker absorptiometer (use Ilford No. 605 filters). Obtain the dissolved oxygen content of the sample from a previously prepared calibration graph.

PREPARATION OF CALIBRATION GRAPH—

Run 0·2, 0·4, 0·6, 0·8 and 1·0-ml portions of 0·001 *N* iodine from a microburette into separate 100-ml calibrated flasks. Dilute each solution to the mark with water containing 0·4 ml each of alkaline iodide solution and diluted sulphuric acid (3 + 1) per litre. Place 1 ml of 3:3′-dimethylnaphthidine solution in each flask, and measure the extinctions as described under "Procedure." Use 100 ml of the diluent solution containing 1 ml of 3:3′-dimethylnaphthidine solution as blank.

Plot a graph of dissolved oxygen against extinction (0·2 ml of 0·001 *N* iodine in 100 ml is equivalent to 0·016 p.p.m. of oxygen). If the distilled water has an "iodine demand," the graph will be displaced from the origin. Move the origin to coincide with this zero, and adjust the graph accordingly.[1]

RESULTS

Many recovery experiments were carried out. The residual oxygen in the sample as collected varied from 0·001 to 0·038 p.p.m. For the first few samples the blank value was not determined in terms of oxygen, the blank solution being used merely as a reference. Later, however, the oxygen equivalent to the blank value was noted as a guide to the variation in reducing matter and also to deterioration of the iodine solution. The results are shown in Table I; it can be seen that agreement between added and recovered oxygen is good.

The precision, as measured by the 95 per cent. limits of error of one determination, is somewhat poorer than that of Potter and White's method,[7] but is adequate for routine work. When less than 0·01 p.p.m. of residual oxygen is present, the precision is within that required for all practical purposes, *viz.*, ±0·002 p.p.m. at the 0·007 p.p.m. level. The maximum error is 0·0032 p.p.m.

TABLE I

RECOVERY OF DISSOLVED OXYGEN

Sample No.	Blank value, as oxygen, p.p.m.	Dissolved oxygen in sample, corrected for blank value— residual, p.p.m.	total, p.p.m.	Dissolved oxygen added, p.p.m.	Dissolved oxygen recovered, p.p.m.	Error, p.p.m.
1	—	0·0384	0·0398	0·0029	0·0014	−0·0015
2	—	0·0013	0·0019	0·0029	0·0006	−0·0023
3	—	0·0010	0·0024	0·0029	0·0014	−0·0015
4	—	0·0013	0·0047	0·0029	0·0034	+0·0005
5	—	0·0073	0·0098	0·0028	0·0025	−0·0003
6	—	0·0038	0·0068	0·0028	0·0030	+0·0002
7	—	0·0019	0·0055	0·0028	0·0036	+0·0008
8	—	0·0070	0·0110	0·0028	0·0040	+0·0012
9	0·0087	0·0173	0·0189	0·0028	0·0016	−0·0012
10	0·0153	0·0060	0·0076	0·0028	0·0016	−0·0012
11	0·0084	0·0095	0·0148	0·0028	0·0053	+0·0025
12	0·0088	0·0128	0·0177	0·0029	0·0049	+0·0020
13	0·0067	0·0117	0·0150	0·0030	0·0033	+0·0003
14	0·0091	0·0113	0·0139	0·0042	0·0026	−0·0016
15	0·0067	0·0157	0·0191	0·0042	0·0034	−0·0008
16	0·0081	0·0153	0·0241	0·0056	0·0088	+0·0032
17	0·0071	0·0182	0·0223	0·0040	0·0041	+0·0001
18	0·0081	0·0108	0·0146	0·0040	0·0038	−0·0002
19	0·0061	0·0041	0·0084	0·0040	0·0043	+0·0003
20	0·0010	0·0075	0·0114	0·0042	0·0039	−0·0003
21	0·0019	0·0066	0·0106	0·0041	0·0040	−0·0001
22	0·0010	0·0024	0·0057	0·0042	0·0033	−0·0009
23	0·0086	0·0152	0·0184	0·0061	0·0032	−0·0029
24	0·0053	0·0119	0·0165	0·0060	0·0046	−0·0014
25	0·0043	0·0080	0·0113	0·0059	0·0033	−0·0026
26	0·0043	0·0074	0·0137	0·0058	0·0063	+0·0005
27	0·0097	0·0064	0·0106	0·0037	0·0042	+0·0005
28	0·0075	0·0135	0·0154	0·0024	0·0019	−0·0005
29	0·0064	0·0055	0·0125	0·0058	0·0070	+0·0012
30	0·0026	0·0113	0·0135	0·0024	0·0022	−0·0002
31	0·0040	0·0020	0·0066	0·0037	0·0046	+0·0009
32	0·0068	0·0017	0·0063	0·0058	0·0046	−0·0012
33	0·0045	0·0086	0·0132	0·0043	0·0046	+0·0003
34	0·0045	0·0080	0·0123	0·0043	0·0043	±0·0000
35	0·0063	0·0110	0·0174	0·0057	0·0064	+0·0007
36	0·0021	0·0121	0·0209	0·0064	0·0088	+0·0024
37	0·0115	0·0058	0·0117	0·0064	0·0059	−0·0005
38	0·0068	0·0219	0·0305	0·0082	0·0086	+0·0004
39	0·0023	0·0115	0·0195	0·0082	0·0080	−0·0002
40	0·0022	0·0084	0·0220	0·0118	0·0136	+0·0018
41	0·0014	0·0111	0·0226	0·0101	0·0115	+0·0014
42	0·0034	0·0050	0·0161	0·0101	0·0111	+0·0010
43	0·0009	0·0096	0·0199	0·0101	0·0103	+0·0002
44	0·0085	0·0129	0·0193	0·0076	0·0064	−0·0012
45	0·0036	0·0042	0·0136	0·0100	0·0094	−0·0006

	All results	Twenty-six results having residual oxygen <0·010 p.p.m.
Mean error, p.p.m.	±0·0010	±0·0009
Standard deviation, p.p.m.	±0·0013	±0·0010
95 per cent. limits of error of one determination, p.p.m.	±0·0026	±0·0021

The values for the blank are sometimes too small, and more oxidant could have been used. The amount of iodine necessary will vary for different boiler-feed systems. To avoid excessive pressure in the sample bottle it is best to maintain the addition of iodine at 0·1 ml

and to use a more concentrated solution if more oxidant is required. It was necessary to renew the 0·005 *N* iodine weekly.

Interference

Potter and White[7] pointed out that serious uncorrected interference with Winkler's reaction occurs if ferrous iron is present and used an ion-exchange column to remove it during sampling. The proposed method is based on Winkler's procedure and is subject to the same interference; ferrous iron should therefore be removed by Potter and White's ion-exchange procedure.

The use of hydrazine as an oxygen scavenger is common in high-pressure boiler-feed systems. Hydrazine interferes seriously with the oxidation of 3:3′-dimethylnaphthidine and invalidates the procedure. However, it is usual to sample for dissolved oxygen upstream of the point at which hydrazine is added, the oxygen level being used as the control for the amount of hydrazine required. Volatile amines, chiefly morpholine and *cyclo*hexylamine, are also frequently added. In my experiments, morpholine did not interfere when the amount was of the normal level used (about 2 p.p.m.).

Conclusions

The proposed method for determining microgram amounts of dissolved oxygen is simple and accurate. The sampling apparatus required is simpler and less expensive than that used by Potter and White, and the method can be used successfully on a routine basis after only a little practice.

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The Spectrophotometric Determination of α-Tocopherol

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A method for the determination of α-tocopherol in oils and foods is described. The principle of the method is (*i*) saponification of the sample and extraction of the unsaponifiable matter, (*ii*) adsorption chromatography on a column of alumina, (*iii*) partition chromatography of the α-tocopherol fraction on strips of paraffin-impregnated paper in a horizontal jar and (*iv*) measurement of the extinction of the α-tocopherol fraction and calculation of the potency by a geometric correction procedure.

The method is specific for α-tocopherol. It has been applied mainly to oils, livers and fillets from fish, for which it has the advantage of simultaneously determining vitamin A. Certain other foods and feed components have been analysed, and the results are discussed. The method is mainly intended for samples containing only α-tocopherol, but it can also be applied to products containing mixed tocopherols.

With the known existence of all seven methyltocols in different concentrations in natural products,[1,2] the determination of total tocopherol is of less interest as a vitamin-E assay.

Although several of the tocopherols can be determined by two-dimensional paper chromatography,[1] a specific and exact determination of α-tocopherol may be of value. Non-α-tocopherols are found mainly in vegetable seeds and foods made therefrom, and the vitamin-E activities so far reported for these compounds are far below that of α-tocopherol (β- and ζ-tocopherol exhibit 40 per cent. of the activity of α-tocopherol; the others exhibit 10 per cent. or less[3,4]). The recent practice of fortifying certain feeds with α-tocopherol further increases the need for a specific method for its determination.

Experimental

The spectrophotometric behaviour of α-tocopherol was investigated by Moore and Rajagopal in 1940,[5] but the low intensity of absorption and the short ultra-violet wavelength of its maximum ($E^{1\%}_{1cm} = 72$ at 292 mμ) have so far made it of little promise.

A combined separation by adsorption and partition chromatography should, however, give an α-tocopherol fraction with a sufficiently specific absorption curve. Two-dimensional chromatography on paper, as proposed by Green, Marcinkiewicz and Watt,[1] is not practical for quantitative elution purposes. This has been discussed by Mason and Jones,[6] who prefer to develop the papers slightly with the Emmerie - Engel reagent before elution, a method not suitable for ultra-violet spectrophotometry. Adsorption chromatography on alumina can be carried out rapidly and without loss; it has the further advantage of providing a simultaneous determination of α-tocopherol and vitamin A[7] (as in fish oils). Bro-Rasmussen and Hjarde[8] found that separation of the tocopherols on a column of magnesium phosphate was sufficient for the subsequent colorimetric determination of α-tocopherol; Edisbury, Gillow and Taylor,[9] who used an alumina column, purified the fraction by passage through slightly alkaline alumina. We have fractionated the unsaponifiable matter from different fish oils and vegetable oils through alumina columns in accordance with the principles described by Gridgeman, Gibson and Savage[10] and have found that, although recovery of α-tocopherol and separation from vitamin A is good, the specificity of the absorption curve is generally insufficient for quantitative spectrophotometry. The use of an alkaline column[9] is hazardous, as a slight excess of alkali can result in high losses of tocopherol. Neither this fractionation nor a second passage through normal alumina suffices for spectrophotometry. We have therefore purified the α-tocopherol fraction from the alumina column by partition chromatography on strips of paraffin-impregnated paper. Strips of paper between 1·0 and 1·5 cm wide are suitable, as they can be rapidly compared with a developed strip and can be cut into small pieces for elution. A relatively large amount of α-tocopherol is needed to obtain a concentration suitable for subsequent spectrophotometric measurement (spots formed by 100 μg of α-tocopherol eluted in 4 ml of ethanol gave extinction values of about 0·200). Such

amounts tend to form streaks on Whatman No. 1 paper, but well defined spots are obtained on the thicker Whatman 3 MM paper. The liquid used for impregnation should preferably be only slightly soluble in ethanol to avoid irrelevant absorption. Medicinal liquid paraffin is suitable, but 2:6:10:15:19:23-hexamethyltetracosane (squalane) is better. Squalane, which has been used as a non-polar phase in gas - liquid chromatography,[11] is a single defined substance having no light absorption in the ultra-violet region. It has the further advantage of almost constant viscosity at different temperatures, which is useful for paper chromatography at elevated or low temperatures (we use the same paper-chromatographic equipment for different procedures requiring different temperatures).

α-Tocopherol is oxidised by air, which should be excluded, if possible, during the application of the spot to the paper. This can be conveniently achieved by horizontal paper chromatography. Meredith and Sammons[12] recommend horizontal paper chromatography and point out the ease with which solvent equilibrium can be attained in the vessel; Roberts[13] recommends it because separation is more rapid than that by descending or ascending-solvent paper chromatography. The technique has the further advantages that the papers can be placed in position before the spots are applied, thereby avoiding further handling, and the vessel can be conveniently placed on shelves in an incubator or refrigerator. When working with unstable compounds, the air in the vessel can be replaced by nitrogen.

We have found that stainless-steel jars can be used as chromatographic vessels. Such jars are commercially produced for the food industry and are therefore cheap and easily obtainable. They are also light in weight and more durable than glass jars. The arrangement of the glass lid and nitrogen inlet is shown in Fig. 1 (*b*).

When 85 per cent. of ethanol in water is used as solvent, the α-tocopherol spot has a mean R_F value of 0·4, vitamin A moves with the solvent front and another tocopherol spot, presumably that of a dimethyltocopherol, sometimes occurs at R_F 0·6. Large amounts of α-tocopherol produce extended spots, *e.g.*, R_F from 0·25 to 0·50, but this seems to have no effect on the absorption curve.

The application of quantitative spectrophotometry depends on the correction of irrelevant absorption,[14] a problem extensively discussed in connection with the determination of vitamin A. The principle of geometric correction is well known and is used in official methods for determining vitamin A.[15,16] It has also been applied to the determination of ergosterol[17] and provitamin D.[18] The steep and symmetrical absorption curve of α-tocopherol in ethanol is suitable for a geometric correction procedure, but it must be stressed that small corrections give the most exact results; corrections of less than 10 to 15 per cent. may be accepted as reliable. Generally, the correction procedure tends to give low values. Our correction formula (see p. 709) was calculated, as described by Oser,[19] from the absorption values in Table I.

The α-tocopherol fractions from fish oils obtained by adsorption chromatography on alumina normally have absorption maxima near 292 mμ. The irrelevant-absorption curve calculated by difference from these fractions has an absorption maximum between 270 and 275 mμ. The extinction value of this absorption is generally low compared with the corresponding α-tocopherol absorption, but one prominent exception has been found. Dogfish-liver oils have an absorption maximum at 282 mμ after chromatography on alumina. This fraction was separated by paper chromatography into three components having R_F values of 0·1 to 0·2, 0·4 and approximately 0·8. The first of these components had the greatest absorption, maximum extinction occurring at 272 mμ, and an absorption curve similar to that of ubiquinone.[20] As the chromatographic behaviour corresponded to that of ubiquinone, we tried to reduce it to tocopherol-like substances, but obtained no fractions giving positive results with Emmerie and Engel's reagents. Further work on this problem is in progress.

Method

Apparatus—

The chromatographic equipment used is shown in Fig. 1. Extinction measurements were made in 1-cm quartz cells with a Beckman DU spectrophotometer (hydrogen lamp).

Reagents—

Ethanol—Distilled from potassium permanganate and sodium hydroxide pellets.

Potassium hydroxide solution, 60 per cent. w/w, aqueous.

Pyrogallol solution, 5 per cent., ethanolic.

Diethyl ether—Freshly distilled from ferrous sulphate.

Light petroleum, boiling range 60° to 80° C—Distilled from sodium hydroxide pellets and then shaken with concentrated sulphuric acid, if necessary.

Alumina ("nach Brockmann")—Shaken with 6 per cent. of water for at least 15 minutes before use.

Ferric chloride solution, 0·2 per cent., ethanolic.

2:2'-Dipyridyl solution, 0·5 per cent., ethanolic.

Acetone.

Squalane solution—Prepare a 10 per cent. solution of 2:6:10:15:19:23-hexamethyltetracosane in light petroleum (the squalane used was obtained from Johan C. Martens and Co. Ltd., Bergen, Norway). A similar solution of medicinal liquid paraffin can be used instead.

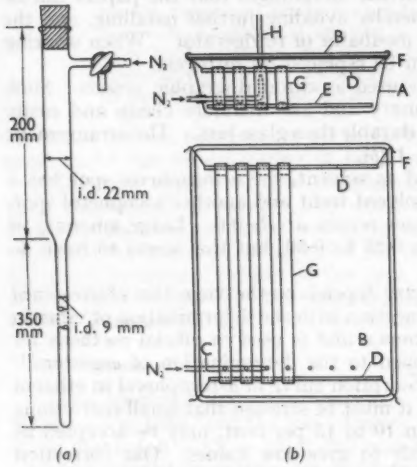

Fig. 1. Equipment for chromatography. (*a*) Column for adsorption chromatography (up to six samples can be run simultaneously). (*b*) Stainless-steel jar for paper chromatography: A, jar (approximately 30 cm × 40 cm × 7 cm); B, glass lid with holes for application of samples to paper strips; C, nitrogen-inlet tube; D, glass tubes for supporting paper strips; E, glass rod for weighting the ends of paper strips to be submerged in eluting agent; F, cotton thread, through glass tubes and fastened to the top of the glass lid by cellulose tape; G, paper strips fastened with paper fasteners; H, capillary pipette for application of samples

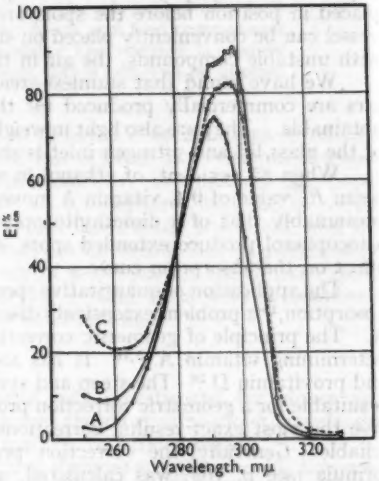

Fig. 2. Ultra-violet absorption spectra measured on preparations of saponified D-α-tocopheryl hydrogen succinate of α-tocopherol in different solvents: curve A, ethanol; curve B, *cyclo*hexane; curve C, chloroform

PROCEDURE—

Heat a sample containing at least 0·5 mg of α-tocopherol in a bath of boiling water for 20 minutes with 3 ml each of 60 per cent. potassium hydroxide solution and ethanolic pyrogallol solution and 25 ml of ethanol. If more than 3 g of oil are needed to obtain sufficient α-tocopherol, saponify two or more portions, and combine them later.

Add 50 ml of water, and extract unsaponifiable matter with one 50-ml and then three 20-ml portions of diethyl ether. Wash the combined extracts with one 50-ml and then four 30-ml portions of water (take care at first to avoid formation of emulsions). Evaporate the extract under reduced pressure on a bath of warm (but not boiling) water, dissolve the residue in a few millilitres of light petroleum, and transfer the solution to a 20-cm column of alumina. (Note that during preparation and subsequent elution the column should never

be allowed to run dry.) Develop the chromatogram with 50 ml of light petroleum, and elute the α-tocopherol fraction with a 4 per cent. solution of ether in light petroleum (the concentration of ether can be increased to 6 per cent. if necessary). Collect the eluate in 10-ml calibrated flasks, and carefully evaporate 0·25 ml of each fraction in a small white porcelain dish (use a current of air). Add 1 drop each of ferric chloride and 2:2′-dipyridyl solutions to the residue in each dish; a carmine colour in the liquid or in a ring on the sides of the dish indicates the presence of α-tocopherol, which should be collected in two to five 10-ml fractions (see Note). If vitamin A is present the fluorescent vitamin-A band should begin at least 5 cm above the base of the column after the α-tocopherol band has been eluted.

Combine 8-ml portions from each of the positive fractions, evaporate under reduced pressure, and dissolve the residue in 2 ml of acetone. Transfer this solution, in portions of 0·1 to 0·5 ml (100 μg), to strips of Whatman 3 MM chromatography paper, 15 mm × 300 mm, that have been washed several times with ethanol, dried, dipped in squalane solution and then dried *in vacuo* (each strip should contain 1·5 mg of squalane). Attach the strips to glass tubes, hang them in the jar, and close the lid. Displace the air in the jar by nitrogen, and apply acetone to the strips with a capillary pipette through the holes in the lid. During this procedure, acetone is continuously evaporated from the paper by a stream of nitrogen, see Fig. 1 (*b*). Include one blank strip and one strip for colour development with each set of papers. Add a solution containing 85 per cent. of ethanol in water, seal the jar with cellulose tape, and allow the papers to develop at room temperature. The solvent front should move between 15 and 20 cm, which takes approximately 4 hours. (If dimethyltocopherols are present it may be desirable to allow development to continue for a further 1 hour.) Remove the papers, suspend them vertically, and apply ferric chloride and 2:2′-dipyridyl solutions to one strip by means of a cotton-wool pad. Mark the positions of the α-tocopherol spots with a pencil on the other strips, cut them out, and then rapidly cut them into small (1 mm) strips. Place these strips in a centrifuge tube containing 4 ml of ethanol, insert a stopper, and shake slowly for 5 minutes. Spin in a centrifuge to remove lint, and transfer the solution to 1-cm quartz cells. The extinctions at 280, 292 and 301 mμ are used for the calculation, but for identification purposes the exact position of the maximum should be established. Calculate the corrected extinction value from the expression—

$$E_{corr.} = 2{\cdot}778\ E_{292} - (1{\cdot}552\ E_{280} + 1{\cdot}626\ E_{301})$$

This value, divided by the extinction found for the standard, gives the α-tocopherol content of the sample.

NOTE—When the amount of non-α-tocopherols present is greater than that of α-tocopherol, good separation may be difficult. However, by using a tightly packed column and a slow rate of elution it should be possible to collect at least one tocopherol-free 10-ml fraction between the two tocopherol-containing fractions.

RESULTS

The proposed method has been in constant use for the last 2 years in our laboratory. D-α-Tocopheryl hydrogen succinate was chosen as a standard preparation, as proposed by Joffe and Harris.[21] This is stable, easy to handle and has given reproducible results. After chromatography on alumina, its extinction has varied between 74 and 76, which has been taken as 100 per cent. recovery. Fig. 2 shows the absorption curves of this product in three solvents, and the results needed to calculate the correction formula are shown in Table I. The paper-chromatographic step has given recoveries between 92 and 98 per cent., the mean loss being 5 per cent. This loss apparently arises from the partition of α-tocopherol between the squalane in the paper and the ethanol used as extraction solvent. Complete recovery can be obtained by extraction with acetone, but as this causes higher blank values we prefer to use ethanol. Recovery by the proposed method has also been tested by adding known amounts of standard to the sample before saponification; over-all reproducibility is to within ±5 per cent.

The method was applied mainly to fish oils, which have repeatedly been shown to contain α-tocopherol. Jansen and Kringstad[22] found α-tocopherol contents of 33 to 130 μg per g in herring oil and 23 μg per g in whale oil. Robeson and Baxter[23] prepared α-tocopherol from shark-liver oil, and Lieck and Willstaedt[24] found α-tocopherol contents of 18 μg per g in turbot liver and 10·5 μg per g in fillets of Baltic Sea herring. Brown,[25] by chromatography on petroleum jelly coated paper, found α-tocopherol contents between 250 and 450 μg per g in fish-liver oils. Our results, which are all in the same range as those just mentioned, are

shown in Table II. Greenland shark-liver oil is of special interest; it gave values up to 700 μg of α-tocopherol per g. The results for fish oils are in the same range as those for vegetable oils (from 60 μg per g in soya-bean oil to 600 μg per g in cottonseed oil). The low vitamin-E activity shown by fish oils in spite of their α-tocopherol content has been discussed by Moore, Sharman and Ward.[26]

TABLE I

VALUES OF $E^{1\%}_{1cm}$ FOR α-TOCOPHEROL IN THREE SOLVENTS

Wavelength, mμ	Extinction in— ethanol	*cyclo*hexane	chloroform
250	2·9	10·4	28·5
255	2·0	9·1	22·9
260	3·6	9·6	20·9
265	8·2	12·4	21·2
270	16·3	18·7	24·9
275	28·2	30·0	32·7
280	44·5	45·6	45·0
285	60·5	66·0	61·0
290	72·5	83·9	76·2
291	73·7	86·4	78·0
292	74·5	86·6	79·4
293	73·8	86·9	80·4
294	72·9	87·4	81·1
295	70·9	88·0	81·8
296	68·5	89·7	82·3
297	65·0	89·7	82·3
298	61·1	90·6	81·7
299	56·9	89·3	80·4
300	52·2	84·8	77·8
301	46·6	80·5	74·5
310	5·4	9·3	15·6
320	0·0	0·8	1·9
330	0·0	0·8	1·2

Table II also shows the amounts of α-tocopherol found in some other food products. These analyses show that the proposed method is reliable for most products containing only α-tocopherol, and the determinations in cod fillets and bacon fat are instances in which sufficiently specific absorption curves were obtained at α-tocopherol contents of a few micrograms per gram. Grass meal and kelp meal (*Laminaria sp.*, which contains only α-tocopherol[27]) were further samples for which the method gave accurate results, but for egg yolk and butter the correction was up to 25 per cent. and the results were consequently less accurate.

TABLE II

AMOUNTS OF α-TOCOPHEROL FOUND IN CERTAIN OILS AND FOODS

Sample	Number of samples analysed	α-Tocopherol content, μg per g
Cod-liver oil	10	180 to 225
Dogfish-liver oil	5	150 to 350
Greenland shark-liver oil	3	300 to 700
Cod liver	1	100
Haddock liver	3	0 to 125
Coalfish liver	2	90 to 150
Herring liver	1	50
Herring oil	2	40 to 60
Catfish liver	1	300
Caplin oil	1	230
Herring fillet	1	3
Cod fillet	1	2
Olive oil	1	220
Ground-nut oil	2	110 to 140
Grass meal	1	80
Kelp meal	1	30
Dried vitamin preparation for addition to feeds	1	500
Egg yolk	4	16 to 39
Bacon fat	10	2 to 15
Butter	2	18 to 25
Margarine	4	40 to 60

Foods containing several tocopherols must be analysed with great care to obtain reliable results. Most vegetable oils, as well as margarine,[28] can be analysed with reasonable corrections of the absorption curves, but mixed feeds, grains and seeds cannot be accurately analysed without further modification to the method. Such products, however, should be analysed for non-α-tocopherols also, as the presence of these homologues, despite their low vitamin-E activity, may substantially increase the total activity of some samples. Mixed feeds containing added α-tocopherol, *e.g.*, as acetate, can be analysed by the proposed method, as such products have a high potency.

It is unfortunate that no method is known for distinguishing between the D and DL forms of α-tocopherol, as these forms differ by about 30 per cent. in biological activity.[29]

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The Determination of Small Amounts of Tin

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A colorimetric method based on optical-density measurements on solutions of a hitherto unreported green reduction compound of silicomolybdic acid has been developed for determining small amounts of tin. The method was required particularly for determining organo-tin compounds in textiles. Amounts from 0·5 to 1·25 mg of tin can be determined with an accuracy to within ±2 per cent., and amounts as small as 0·05 mg can be determined to within ±4 per cent.

LABORATORY and industrial tests have shown that organo-tin compounds are effective fungicides in textiles and paints[1,2,3]; concentrations as low as 0·1 per cent. are adequate in some circumstances. A rapid and accurate method for determining the concentration of such compounds in treated materials is essential for a thorough study of their value as fungicides and also for the control of commercial processes.

The general formula of these compounds is R_3SnX, in which R is either alkyl or aryl and X is a negative ion, *e.g.*, acetate, halide or oxide. The determination of tin is the easiest method for quantitative analysis, especially as textiles are rarely treated with tin compounds (except for a few fire-proofing agents that are now obsolete). Organo-tin compounds contain from 30 to 50 per cent. of tin, and the amount of tin usually found in a 1-g sample of fabrics rot-proofed with such compounds is between 0·3 and 2·5 mg. A volumetric or gravimetric procedure for determining the tin content of the fabric is therefore not convenient.

Baker, Miller and Gibbs[4] described the determination of tin by a colorimetric method involving a blue reduction compound of silicomolybdic acid, but I found that consistent results having the required accuracy could not be obtained by their method. Several reasons were found for the lack of reproducibility and are discussed later; a major cause was that, under the conditions of the determination, several silicomolybdous acids may be formed. Strickland[5] stated that silicomolybdic acid exists in at least two forms, which he called α and β. These acids were formed in different proportions depending on the conditions, and the β-form slowly changed spontaneously to the α-form. Further, Strickland stated that he obtained two reduction products from the α-acid by using 4 and 5 gram-equivalents of stannous chloride per mole of silicomolybdic acid, but only one reduction compound was formed from the β-acid (when 5 gram-equivalents of stannous chloride were used).

The chemistry of the reduction of the silicomolybdic acids is, however, even more complicated than this. Emerald-green reduction compounds of the α- and β-acids are formed when up to approximately 0·5 gram-equivalent of stannous chloride is added and are definite and distinct compounds. These reduction complexes do not correspond to Strickland's "sea-green" α-silicomolybdous acid, since they are formed much earlier in the reduction. The green complex of the β-acid has a flat peak in its absorption spectrum at 6800 A; it is remarkably stable in comparison with the blue compound formed in the method described by Baker, Miller and Gibbs,[4] which fades markedly after 5 minutes, especially when the amount of tin present is about 1 mg in 130 ml of reagent solutions. The green compound is therefore considered to be the most suitable complex for use in determining tin. The nature of the reduction products will be discussed fully elsewhere.

EXPERIMENTAL

Because the emerald-green silicomolybdous acid is formed with only 0·5 gram-equivalent of stannous chloride, whereas 5 gram-equivalents are needed to form the blue compound, ten times as much silicomolybdic acid is required for a given amount of tin. The specific extinction coefficient of the green silicomolybdous acid is lower than that of the blue compound, and the green form must therefore be used at higher concentrations in order to obtain optical-density values suitable for measurement.

When the optical densities at 6800 A for solutions obtained by reducing β-silicomolybdic acid with stannous chloride were plotted against concentration of tin, the graph was linear

for amounts of tin from 0·0 to 1·5 mg in 200 ml of final solution. Values lower than theoretical were obtained for amounts of tin greater than 1·5 mg, owing to formation of the blue reduction complex, which has a different absorption maximum.

ACID CONCENTRATION AND REDUCTANT—

In Baker, Miller and Gibb's method, silicomolybdic acid is added to a solution of stannous chloride containing undissolved zinc that is still evolving nascent hydrogen. Even if the liquid is decanted within the 10 seconds specified, the reduction of silicomolybdic acid by nascent hydrogen leads to variable results and appreciable blank values, which become greater when higher concentrations of silicomolybdic acid are used. This effect is avoided in the proposed method by completely dissolving the reductant and then boiling the solution for at least 5 minutes to expel all hydrogen.

The hydrochloric acid concentration must be restricted, as it was found that if the concentration was greater than 2 *N* when the silicomolybdate reagent was added, the intensity of the green colour was decreased. At lower acid concentrations, evolution of hydrogen from zinc was slow, and aluminium, which dissolved more rapidly and produced smaller bubbles, was a more efficient reductant. It was necessary to use commercial-grade aluminium containing 0·3 per cent. of iron; the slight colour produced in blank solutions by the iron was constant when approximately equal amounts of aluminium were used as reductant.

STABILITY OF REAGENTS, SILICOMOLYBDIC ACID AND REDUCTION PRODUCT—

Stock solutions of sodium metasilicate and sodium molybdate are completely stable and can be stored for long periods. It was found that formation of silicomolybdic acid from these solutions was complete within 5 minutes, but the yellow colour of the silicomolybdic acid began to fade when the solution was set aside for longer than 10 minutes. This leads to a systematic error unless a time limit is imposed, after which a fresh solution must be prepared. A similar observation was made by Volk and Weintraub.[6] Attempts to overcome this fading were completely unsuccessful, and the silicomolybdic acid was therefore freshly prepared and used only within 5 to 10 minutes of preparation.

Strickland has pointed out that a molybdate species formed only in moderately acid solution can be reduced to form molybdenum blue when added to stannous chloride in strongly acid solutions. To avoid the possible formation of molybdenum blue in the proposed method, an excess of sodium metasilicate solution is used to ensure that all molybdate is present as silicomolybdic acid.

INTERFERING METALS—

The method is not specific for tin; iron, antimony and possibly any metal that can be reduced by hydrogen will produce the same colour with silicomolybdic acid. However, by distillation from a mixture of hydrobromic and sulphuric acids, tin can be quantitatively separated from all interfering metals except arsenic, antimony and mercury.[7] Alternatively, the organo-tin compounds can be separated from most interfering metals by solvent extraction.

OXIDATION OF SAMPLES—

Organo-tin compounds are generally resistant to normal oxidation procedures, and serious losses occur if the oxidation is prolonged, as some of the compounds are volatile in steam.[8] The most satisfactory method is that described by Kocheshkov,[9] which consists in wet oxidation by a mixture of equal volumes of fuming nitric and fuming sulphuric acids. This mixture rapidly oxidises any organo-tin compounds and also digests the textile present.

METHOD

REAGENTS—

Aluminium pellets—The pellets used had a purity of approximately 99·5 per cent. and were tin-free; each weighed 0·03 to 0·04 g.

Nitric acid, fuming—Analytical-reagent grade.

Sulphuric acid, fuming—Analytical-reagent grade.

Hydrochloric acid, sp.gr. 1·18—Analytical-reagent grade.

Sulphuric acid, sp.gr. 1·84—Analytical-reagent grade.

Sodium molybdate solution—Dissolve 29·1 g of analytical-reagent grade sodium molybdate, $Na_2MoO_4.2H_2O$, in 800 ml of distilled water, add 20 ml of sulphuric acid, sp.gr. 1·84, cool, and dilute to 1 litre.

Sodium metasilicate solution—Dissolve 25·4 g of sodium metasilicate, $Na_2SiO_3.5H_2O$, in 800 ml of distilled water, and dilute to 1 litre.

Silicomolybdate reagent solution—By pipette, place 100 ml of sodium molybdate solution in a 1-litre calibrated flask. Add 50 ml of sodium metasilicate solution with constant mixing, and dilute immediately to the mark. This solution must be used within 5 to 10 minutes of preparation.

Standard tin solution—Dissolve 0·05 g of 99·99 per cent. pure metallic tin in 1 litre of 50 per cent. v/v hydrochloric acid. This solution contains 0·05 mg of tin per ml.

PREPARATION OF CALIBRATION GRAPHS—

For 0·25 to 1·5 mg of tin—Take suitable aliquots of standard tin solution to cover the range 0·25 to 1·5 mg of tin, add hydrochloric acid, sp.gr. 1·18, and dilute each solution to 100 ml with water. (The solutions should now be approximately, but not greater than, 2 *N* in total acid.) Add one aluminium pellet to each solution, and place on a hot-plate adjusted so that the solution boils just before the aluminium has completely dissolved. Continue to boil for 5 minutes to expel hydrogen, and then rapidly add to each solution 100 ml of silicomolybdate reagent solution. Cool the solutions to 20° C in a water bath, and dilute each with distilled water to 200 ml in a calibrated flask.

After 15 minutes, measure the optical densities of the solutions against distilled water at 6800 A in 3·5-cm cells with a Uvispek spectrophotometer. Also measure the optical density of a blank solution that has been treated in the same way as the standards, and subtract this value from the optical densities of the standards. Plot a graph of optical density against amount of tin present. The graph is linear from 0·0 to 1·5 mg of tin and has the equation $y = 0{\cdot}625x$, where y is the optical density in a 3·5-cm cell and x is the amount of tin present in milligrams. Under these conditions, the optical densities of solutions containing more than 1·25 mg of tin are too high for precise measurement and those of solutions containing less than 0·5 mg of tin are too low.

For 0·05 to 0·30 mg of tin—Proceed exactly as described above, but use only one-fifth of the amounts stated. For example, add 20 ml of silicomolybdate reagent solution, and dilute the final solution to 40 ml. Under these conditions, the graph is similar, but has the equation $y = 0{\cdot}125x$. (The complete range of tin that could be determined was not fully investigated, since the method was originally developed for amounts up to 1·5 mg.)

PROCEDURE—

Take a weight of fabric such that, when digestion has been completed and the resulting solution diluted to 250 ml, a suitable aliquot contains between 0·5 and 1·25 mg of tin. Cut the sample into pieces, place in a Kjeldahl flask, and carefully add not more than 15 ml of a mixture of equal volumes of fuming nitric and fuming sulphuric acids. Slowly warm the flask, and, after the initial vigorous reaction has subsided, add small amounts of concentrated nitric acid, and heat the flask until the solution is clear. Continue to heat until fumes of sulphur trioxide are evolved, cool, carefully dilute with distilled water, and re-heat until fumes of sulphur trioxide are evolved to remove all traces of nitric acid. Cool, and dilute with distilled water to 250 ml in a calibrated flask. Take a suitable aliquot of the solution, and continue as described for preparing the calibration graphs.

Note that an alternative procedure can be used for organo-tin compounds that are soluble in a suitable solvent. The sample is heated under reflux with the solvent for 5 hours in a Soxhlet extractor, and, after the solvent has been evaporated, the residue is oxidised in the normal way. This procedure has the advantages that the organo-tin compound is separated from interfering metals and that it is not necessary to oxidise the entire sample; it is limited only by the availability of a suitable solvent. (The amounts of tin found in cotton and flax that had been treated with tri(*n*-butyl)tin oxide were the same by both procedures; acetone was used as solvent in the alternative method.)

Discussion of Results

Table I shows the recovery of pure metallic tin by the proposed method. The accuracy of the results in the range 0·5 to 1·25 mg of tin, *i.e.*, ±2 per cent., is well within the accepted limits of accuracy for a colorimetric determination. This is attributed to the fact that optical-density measurements are made in the most accurate region and to the fairly high sensitivity of the developed colour. The larger error in the determination of 1·6 mg of tin arises because the calibration graph is not linear for amounts of tin greater than 1·5 mg. It can be seen from Table I that the error in the determination of 0·21 mg of tin is relatively large; this is because the intensity of the colour developed is low and instrumental measurement is hence more uncertain. When a final volume of 40 ml was used the accuracy attained (to within ±4 per cent.) is considered to be excellent for an amount of tin as small as 0·05 mg.

In Table II the amounts of tin found by the proposed method in two organo-tin compounds are compared with those found gravimetrically, as stannic oxide, and with the theoretical tin contents of the pure compounds. It can be seen that agreement between the colorimetric and gravimetric results is good.

The desired degree of accuracy could be attained by carrying out duplicate determinations. It is considered that the proposed method is more rapid and more precise than other methods for determining similar amounts of tin; three determinations in duplicate can be carried out simultaneously in 45 minutes after oxidation or extraction.

Table I

Recovery of Metallic Tin by the Proposed Method

Tin present, mg	Tin recovered, mg	Error, %
0·050	0·050*	0·0
	0·052*	+4·0
	0·048*	−4·0
0·100	0·098*	−2·0
	0·100*	0·0
	0·104*	+4·0
0·211	0·201	−5·3
	0·204	−3·3
	0·206	−2·4
0·528	0·520	−1·5
	0·498	−5·8
	0·530	+0·4
0·845	0·855	+1·2
	0·846	+0·1
	0·840	−0·6
1·056	1·078	+2·1
	1·050	−0·6
	1·062	+0·6
1·267	1·258	−0·7
	1·270	+0·2
	1·260	−0·5
1·659	1·550	−6·6
	1·530	−7·8
	1·540	−9·6

* Determined by using one-fifth of the normal amounts of reagents and diluting the final solution to 40 ml.

Table II

Comparison of Results by Colorimetric and Gravimetric Methods

Compound	Theoretical tin content of pure compound, %	Tin found by proposed method, %	Tin found by gravimetric method, %
Tri(*n*-butyl)tin fluoride	38·35	37·00	37·40
Tri(phenyl)tin fluoride	32·16	31·30	31·35

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The Simultaneous Spectrophotometric Determination of Copper and Nickel in Low-alloy Steels

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A spectrophotometric procedure is described for the simultaneous determination of copper and nickel, both present to the extent of 1 per cent. or less, in low-alloy steels. The colorimetric reagents used are bis*cyclo*hexanone-oxalyldihydrazone for copper and disodium ethylbis(5-tetrazolylazo)acetate for nickel.

When the alloy has been dissolved, the solution is buffered and solutions of the two reagents are added. The optical density of the resulting solution is measured at two wavelengths and the amounts of copper and nickel present are determined by reference to a calibration graph. Several British Chemical Standard alloys have been analysed by this procedure; the results show a coefficient of variation of approximately 2 per cent.

The purpose of the work described was to develop a simple procedure for determining copper and nickel, both present in amounts of the order of 1 per cent. or less, in low-alloy steels by making spectrophotometric measurements on a single solution at two wavelengths. With the aim of increasing the efficiency of the analysis of such alloys, similar procedures have been described for chromium and manganese[1] and for molybdenum, titanium and vanadium.[2]

It is preferable in such analyses to allow one reagent to react with both or all of the elements being determined, and on these lines procedures have been developed in which rubeanic acid,[3] its derivatives[4] and related compounds[5] were used for the simultaneous determination of copper, nickel and cobalt in various aluminium alloys and in ferrosilicon. The inherent disadvantages of these reagents are (*a*) poor selectivity, (*b*) the necessary presence of dispersing agents and (*c*) the fact that the absorption curves of the respective metal complexes overlap considerably; the last-named disadvantage inevitably leads to poor reproducibility. The search was therefore extended to include individual reagents for copper and nickel, with the qualification that the wavelengths of peak absorption of the coloured complexes were widely separated and the corresponding background absorptions were low.

Nioxime[6] was first considered as a reagent for nickel, since it can be rendered selective and, unlike dimethylglyoxime, does not require the presence of oxidising agents. However, it has the disadvantage that dispersing agents are required, and interference from ferric iron can be satisfactorily minimised only by using citric acid as a masking agent.

A second reagent for nickel is disodium ethylbis(5-tetrazolylazo)acetate.[7] This reagent also reacts with cobalt, which does not interfere, and with copper. The colour formed with copper has an absorption peak at a wavelength close to that of the colour formed with nickel, and interference from copper must be overcome.

Of the reagents in current use for copper, two are highly specific; these are 2:9-dimethyl-1:10-phenanthroline (neocuproine)[8,9] and bis*cyclo*hexanoneoxalyldihydrazone.[10] Procedures based on the use of the former have invariably incorporated a solvent extraction of the copper complex, although this complex is soluble in water and has been used without extraction in many determinations. The latter reagent has been used successfully for the direct determination of copper in steels,[11] and, since it is much more sensitive than neocuproine, its use was considered first.

Experimental

Preliminary tests—

Solutions containing bis*cyclo*hexanoneoxalyldihydrazone and copper, together with nickel and nioxime or disodium ethylbis(5-tetrazolylazo)acetate, were used in a series of preliminary tests to determine the compatibilities of the various coloured complexes. These tests showed that the blue colour of the copper complex faded rapidly in presence of the nickel - nioxime complex, but not in presence of any of the individual reagents. Further work, therefore, centred on the use of disodium ethylbis(5-tetrazolylazo)acetate. Measurements for the complex formed by nickel with this reagent were made at 510 mμ and those for the copper - bis*cyclo*hexanoneoxalyldihydrazone complex at 600 mμ, at which wavelengths the reciprocal absorptions were low. The stability constants of the copper complexes with disodium ethylbis(5-tetrazolylazo)acetate and bis*cyclo*hexanoneoxalyldihydrazone are such that formation of the latter prevents formation of the former when a solution of disodium ethylbis(5-tetrazolylazo)acetate is subsequently added.

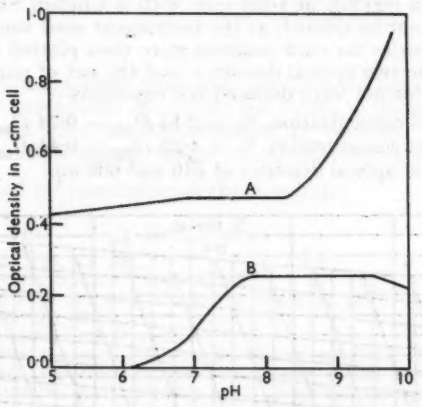

Fig. 1. Effect of pH on colour formation in solutions containing 100 μg of metal per 100 ml: curve A, nickel - disodium ethylbis(5-tetrazolylazo)acetate complex measured at 510 mμ; curve B, copper - bis*cyclo*hexanoneoxalyldihydrazone complex measured at 600 mμ

The variations in optical density with pH for the copper - bis*cyclo*hexanoneoxalyldihydrazone and the nickel - disodium ethylbis(5-tetrazolylazo)acetate complexes were separately determined in solutions buffered by adding ammonia solution and citric acid (see Fig. 1), and it was apparent that the pH range 7·8 to 8·3 was suitable for simultaneous colour formation. The adherence to Beer's law of solutions of the two complexes was separately confirmed in the range required, *viz.*, 0 to 100 μg of each metal in a volume of 100 ml.

Details previously published[7,10] indicated that interference from the respective concentrations of other elements likely to be present would be negligible. This was confirmed for cobalt, molybdenum, titanium, vanadium, tungsten, manganese, chromium and silicon by measuring the optical densities of solutions containing either copper and bis*cyclo*hexanoneoxalyldihydrazone or nickel and disodium ethylbis(5-tetrazolylazo)acetate and the various other metals in the pH range 7·8 to 8·3. However, at a concentration of 9·5 mg per 100 ml of final solution, ferric iron had a slight effect, the measured optical densities at 510 and 600 mμ being increased by additional absorption from the iron to the extent that the values for copper and nickel in a sample containing 95 per cent. of iron, for which allowance was not made, would be 0·01 and 0·04 per cent. high, respectively. The necessity for making measurements against a blank solution containing 9·5 mg of iron per 100 ml was therefore apparent, although the error incurred for steels having iron contents a few per cent. above or below this value would clearly be insignificant.

After this preliminary work, a calibration net was prepared.

PREPARATION OF CALIBRATION NET—

A series of solutions covering the range 0 to 1 per cent. of both copper and nickel was prepared from solutions of the two metals. The values used corresponded to 0·0, 0·25, 0·50, 0·75 and 1·00 per cent. of each metal and combinations thereof, a total of twenty-five solutions. Each solution contained 9·5 mg of pure iron dissolved in 10 ml of 25 per cent. v/v sulphuric acid, to which 10 ml of 40-volume hydrogen peroxide were added. The solutions were then boiled to remove the excess of hydrogen peroxide and each was diluted to 200 ml. To a 10-ml portion of each of these solutions were added 5 ml of an approximately 20 per cent. w/v solution of ammonium citrate adjusted to pH 10·0 ± 0·1 by adding ammonia solution, 2 ml of a 0·1 per cent. w/v solution of bis*cyclo*hexanoneoxalyldihydrazone in ethanol - water mixture (1 + 1) and 5 ml of a 0·002 *M* aqueous solution of disodium ethylbis(5-tetrazolylazo)acetate. All solutions were diluted to 100 ml, their pH values than being between 7·9 and 8·2, and the optical densities, relative to the solution containing no nickel or copper, were measured at 510 and 600 mμ in 1-cm cells with a Unicam SP600 spectrophotometer. (Slit-width tolerances cannot be quoted, as the instrument used was without means of indication.) The optical densities for each solution were then plotted on a graph, the axes of which were in terms of the two optical densities, and the net of parallel lines was drawn as shown in Fig. 2. From this net were deduced the equations—

$$\text{Nickel concentration, \%} = 2{\cdot}15\ D_{510} - 0{\cdot}74\ D_{600}$$
$$\text{Copper concentration, \%} = 4{\cdot}29\ D_{600} - 0{\cdot}44\ D_{510}$$

where D_{510} and D_{600} are the optical densities at 510 and 600 mμ.

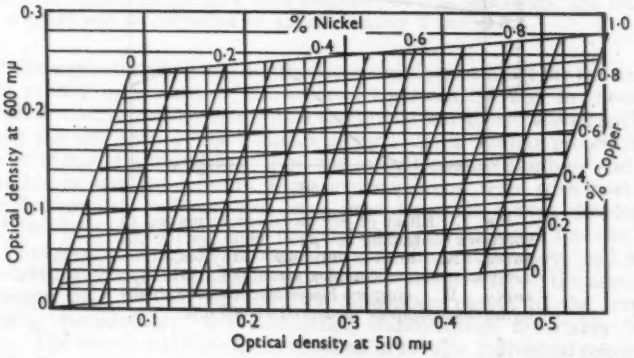

Fig. 2. Calibration net

METHOD

PROCEDURE—

Dissolve 0·20 g of sample in a mixture of 10 ml each of 25 per cent. v/v sulphuric acid and 40-volume hydrogen peroxide. Boil to evolve excess of hydrogen peroxide, cool, and

remove any insoluble matter by filtration through a Whatman No. 40 filter-paper. Wash the filter-paper thoroughly, add the washings to the filtrate, and dilute to 200 ml. To a 10-ml portion of this solution add 5 ml of an approximately 20 per cent. w/v solution of ammonium citrate (adjusted to pH 10·0 ± 0·1 by adding ammonia solution), 2 ml of a 0·1 per cent. w/v solution of bis*cyclo*hexanoneoxalyldihydrazone in 50 per cent. v/v ethanol and then 5 ml of a 0·002 *M* aqueous solution of disodium ethylbis(5-tetrazolylazo)acetate. Dilute to 100 ml, and measure the optical densities of this solution against a blank solution similarly prepared from 0·190 g of pure iron at 510 and 600 mμ in 1-cm cells with a suitable spectrophotometer. Determine the percentages of copper and nickel in the sample by reference to a previously prepared calibration net.

TABLE I

RESULTS BY THE PROPOSED METHOD FOR LOW-ALLOY STEELS

Sample	Copper present, %	Nickel present, %	Copper found, %	Nickel found, %
B.C.S. No. 255 ..	0·240	0·565	0·23, 0·23, 0·245, 0·245, 0·225, 0·225, 0·23, 0·23, 0·23, 0·24, 0·23, 0·24	0·58, 0·57, 0·58, 0·58, 0·575, 0·58, 0·58, 0·575, 0·57, 0·57, 0·575, 0·57
B.C.S. No. 256 ..	0·230	0·185	0·23, 0·235, 0·23, 0·23, 0·23, 0·23, 0·23, 0·23, 0·23, 0·23, 0·23, 0·23	0·19, 0·195, 0·19, 0·19, 0·195, 01·95, 0·19, 0·195, 0·19, 0·19, 0·19, 0·19
B.C.S. No. 257 ..	0·305	0·840	0·30, 0·30, 0·30, 0·30, 0·30, 0·305, 0·30, 0·30, 0·30, 0·30, 0·30, 0·30	0·83, 0·83, 0·83, 0·835, 0·84, 0·835, 0·83, 0·83, 0·83, 0·83, 0·83, 0·83
B.C.S. No. 258 ..	0·185	0·048	0·18, 0·18, 0·18, 0·18, 0·205, 0·195, 0·18, 0·195, 0·18, 0·195, 0·195, 0·195	0·052, 0·052, 0·054, 0·050, 0·050, 0·052, 0·050, 0·050, 0·054, 0·050, 0·050, 0·050

RESULTS

Four suitable samples of low-alloy steels from the British Chemical Standard range were analysed by the proposed procedure; the results are shown in Table I. Accuracy and precision are good, the general level for the coefficient of variation being 2 per cent. The procedure is simple, and a single sample can be analysed in 20 minutes.

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The Determination of Micro Amounts of Dissolved Oxygen in Water

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The determination of dissolved oxygen in the range 0·0 to 0·050 p.p.m. is described. The apparatus used incorporates certain novel features, including an arrangement that allows the reagents used in the experiments to be rendered oxygen-free before the determination is made.

The standardisation of the apparatus in the absence of reducing agents is described.

The mean of the differences between the oxygen added and the oxygen found is nil and the standard deviation of these differences is 0·0018 p.p.m.

In recent years the increased use of high pressures and temperatures and the associated high ratings of boiler heat-transfer surfaces have led to renewed attention being directed to the dissolved-oxygen content of boiler-feed water, as the presence of dissolved oxygen under these conditions greatly increases the risk of corrosion.

It is customary in boiler-house practice to determine dissolved oxygen by chemical methods, although reliable and sensitive physical methods are now coming into use. Most of the chemical methods used are basically modifications of Winkler's technique[1] and permit dissolved-oxygen contents below 0·050 p.p.m. to be accurately determined. These modifications include preparation of a blank to allow for the presence of reducing agents, corrections for dissolved oxygen introduced with the reagents and the development of precise techniques for accurately determining the iodine liberated during the reaction.

Little difficulty is experienced in accurately determining dissolved oxygen in pure water by these methods. However, oxygen scavengers, such as sodium sulphite or hydrazine, are frequently added to high-pressure boiler feed water, and in the presence of these reducing agents the reliability of the usual chemical technique is doubtful. Because of this situation, work began in this laboratory late in 1954 with the object of establishing the relative merits of the various analytical methods in common use. To a certain extent this work has been found to parallel that recently reported by Potter[2] and his associates.

The apparatus described here permits oxygen-free water to be obtained, and, by injecting small amounts of air-saturated water, samples of water having a known dissolved-oxygen content in the required range can be prepared. A comparison between the oxygen measured by a particular method and the true oxygen content gives a measure of the reliability of that method. To assess the accuracy attainable with the apparatus, work began on the determination of dissolved oxygen in pure water, *i.e.*, distilled water that had been passed through an analytical-reagent grade mixed-bed resin. The method recommended by the American Society for Testing Materials[3] was selected as the basis for this initial standardisation.

Description of apparatus

In the normal A.S.T.M. procedure the sampling vessels are flushed ten times with the test solution to obtain a correct sample. This would entail the de-aeration of at least 10 litres of water by boiling and passing nitrogen through it. As it was not desired to have a continuous supply of hot de-mineralised water, a considerable time would have been necessary to heat 10 litres of water to boiling-point. A McLean-type sampling tube[3] has been modified[4] to introduce the reagents through a double oblique-bore three-way tap. However, it was found that the reaction between the sulphuric acid and the alkaline iodide solution in the bore of the tap produced free iodine, but the replacement of sulphuric acid by phosphoric acid seemed to overcome this. Potter[2] discussed the question of sampling tubes and recommended the use of a tube in which the reagent entry was completely washed out after each addition. Other workers[5,6] have used the submerged-bottle technique to add the reagents.

In view of these factors, it was decided to design an apparatus in which the reagents could be added through separate entries, thereby avoiding the possibility of reactions between concentrated reagents. It was also decided that only 2 litres of water should be used in a

single determination and that 500-ml samples should be taken. The apparatus is shown diagrammatically in Fig. 1; a glass float allows the volume of solution in the reaction vessel to be increased when the reagents are run in, but, because of the small clearance, minimises the possible diffusion of oxygen from the solution being tested to the nitrogen above it. The diffusion of reagents and air-saturated water into the test solution is reduced by using capillary tubing for the connections to the reaction vessel.

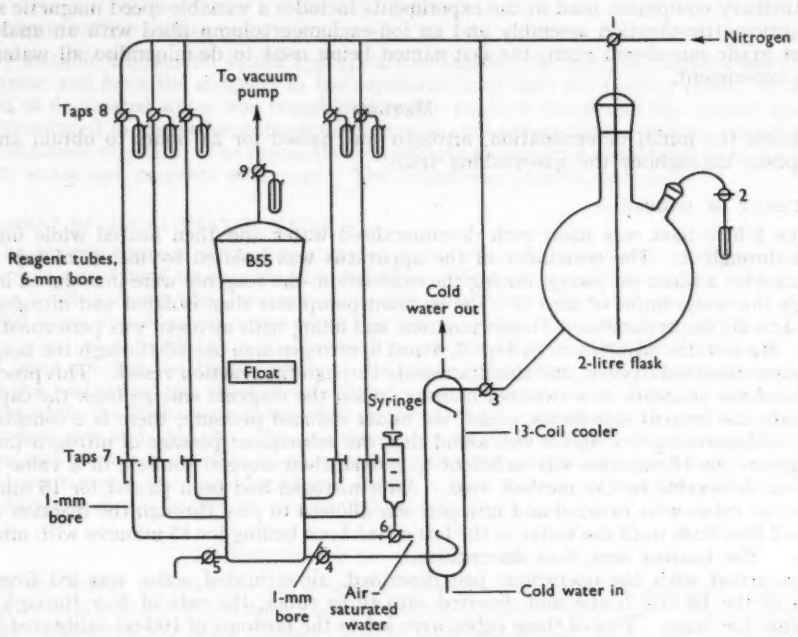

Fig. 1. Diagramatic representation of apparatus

The final consideration in the design of the apparatus was to carry out all the operations, de-aeration of water and reagents, transfer of water to reaction vessel and addition of air-saturated water and reagents, by manipulation of stopcocks, without the need to move pieces of the apparatus from one position to another.

The apparatus consists essentially of a round-bottomed 2-litre flask, used for de-aeration, and a cylindrical reaction vessel, to which the water is transferred after de-aeration. Attached to the reaction vessel are side-limbs having an internal diameter of 1 mm. A syringe is connected to one of these side-limbs, through which an appropriate amount of air-saturated water can be injected to give the desired dissolved oxygen content. Reagent tubes are fitted to the other side-limbs, so that, after dissolved oxygen has been removed by bubbling nitrogen through them, the reagents can be introduced into the reaction vessel through different tubes. The reaction vessel contains the stirring-bar of a magnetic stirrer.

The disposition of the reagent tubes is not truly represented in Fig. 1, the main difference being in the location of the reagent tubes and their allied connecting tubes. These 1-mm connecting tubes have similar dimensions and are located so that each reagent tube is about 1½ inches from the reaction vessel; tap 6 is about 1 inch from the reaction vessel. Four of the connecting tubes are placed at right angles to one another and about 5 inches from the bottom of the reaction vessel. The remaining tube and tap 6 are placed 2½ inches from the bottom of the vessel, at right angles to one another, and off-set from the upper level of connecting tubes. A small thermometer is placed inside the tube connecting the cooler to the reaction vessel. When the apparatus is assembled, the glass ends are butted and poly(vinyl chloride) or vacuum-type rubber tubing is used to cover the joins. Rubber tubing is used when only gas flows in a tube.

The nitrogen (oxygen-free grade) is freed from the last traces of oxygen by passing it through two gas-washing bottles containing 0·1 *M* vanadous sulphate,[7] a wash bottle containing de-mineralised water and finally a heated combustion tube packed with copper gauze.

Air-saturated water is prepared by slowly bubbling clean compressed air through a No. 3 sintered-glass disc inserted at the bottom of a 15-litre aspirator bottle full of water. This water is continually stirred at 20 r.p.m. by a mercury-sealed stirrer.

Ancillary equipment used in the experiments includes a variable-speed magnetic stirrer, a potentiometric-titration assembly and an ion-exchange column filled with an analytical-reagent grade mixed-bed resin, the last-named being used to de-mineralise all water used in the experiment.

Method

Before the initial determination, nitrogen was passed for 24 hours to obtain an inert atmosphere throughout the gas-washing train.

Treatment of sample—

The 2-litre flask was filled with de-mineralised water and then heated while nitrogen flowed through it. The remainder of the apparatus was isolated by means of tap 1 and evacuated by a water-jet pump; during the evacuation, the reagents were introduced in turn through the waste limbs of taps 7. The vacuum pump was then isolated and nitrogen was allowed to fill the apparatus. This evacuation and filling with nitrogen was performed three times. By suitable adjustment of taps 7, 8 and 9, nitrogen was passed through the reagents, to displace dissolved oxygen, and simultaneously through the reaction vessel. This procedure de-aerated the reagents in a two-fold manner; when the reagents emerge from the capillary tubes into the reagent side-necks, which are under reduced pressure, there is a considerable release of dissolved gases, and it was found that the subsequent passage of nitrogen through the reagents for 15 minutes was sufficient to reduce their oxygen content to a value lower than that detectable by the method used. After nitrogen had been passed for 15 minutes, the reagent tubes were isolated and nitrogen was allowed to pass through the reaction vessel and the 2-litre flask until the water in the latter had been boiling for 15 minutes with nitrogen passing. The heating was then discontinued.

Concurrent with the operations just described, air-saturated water was led from the bottom of the 15-litre bottle and diverted into three tubes, the rate of flow through each tube being the same. Two of these tubes were led to the bottoms of 100-ml calibrated flasks and the third was attached to tap 6. The flasks were placed in gas jars and the jars in a large receptable. Air-saturated water was allowed to fill the flasks at least six times and at the same time to flow through the glass syringe via tap 6. Tap 6 was turned until approximately 3 ml of water had flowed into the reaction vessel and was then again turned to permit the air-saturated water to flow through the syringe. The water flow was then terminated, stoppers were placed in the flasks and the plunger of the syringe was inserted. In this way, a uniform sample of water having a dissolved oxygen content corresponding to that contained in the syringe was obtained. In addition, the bore of tap 6 and the capillary connection to the reaction vessel were also filled with this water.

The surplus air-saturated water at the bottom of the reaction vessel was blown out through tap 5 by nitrogen, care being taken that nitrogen continued to bubble through the gas traps of taps 9 and 2. Some de-aerated water was then allowed to flow through the cooling coil into the reaction vessel, subsequently being blown out via tap 5. This operation was carried out three times and freed the reaction vessel of the last traces of air-saturated water. By adjusting the flow rate through the cooling coil, 500 ml of de-aerated water, at 20° C, were transferred to the reaction vessel. The reagent levels were adjusted to the zero marks under nitrogen by altering taps 7 and 8 and allowing the excess of each reagent to flow to waste; the level of water in the syringe was similarly adjusted to the zero mark. During all these operations, a continuous flow of nitrogen was maintained through tap 2.

The magnetic stirrer was switched on and the appropriate amount of air-saturated water was injected. At 3-minute intervals, a 2-ml portion of each reagent was allowed to flow in under nitrogen pressure, and care was taken to add the sulphuric acid slowly to obviate the possibility of releasing iodine from the potassium iodide solution. The order in which reagent solutions were added was potassium hydroxide - potassium iodide - iodine, manganese sulphate and sulphuric acid.

The solution was afterwards run out through tap 5 and the iodine was determined by potentiometric titration. To permit the end-point to be determined in the way described by Hostetter and Roberts,[8] small equal amounts of sodium thiosulphate solution were added and the e.m.f. was read after each addition. During the titration, the burette tip was raised above the solution after each addition, stirring was continuous and the solution was kept under nitrogen. Care was taken during stirring to ensure that no vortex was formed.

Determination of blank value—

By suitable adjustment of the appropriate stopcocks, the nitrogen pressure was allowed to increase and force the reagents in the capillaries back into the reagent tubes. A 500-ml portion of de-aerated water was transferred to the reaction vessel, and the reagent solutions were introduced in the order potassium hydroxide - potassium iodide - iodine, sulphuric acid and manganese sulphate. The precautions to exclude air were not considered to be necessary, as both water and reagents were pure. The iodine was titrated as before.

Treatment of air-saturated water—

The oxygen content of the air-saturated water was determined essentially by means of the sample and blank techniques just described. The concentration of free iodine in the alkaline iodide reagent was, however, increased, as a visual end-point would not have been obtained when starch solution was used, the iodine - iodide concentration used in the 0·0 to 0·050 p.p.m. range being below that needed to produce the blue complex with starch. This would have affected the blank value, as the iodine determined is the algebraic sum of the free iodine added and the effect of interfering ions. Reagents were added by means of 1-ml pipettes while the flasks were submerged in the gas jars, and, after each addition, the flasks were taken out and shaken. The solutions were titrated with 0·1 *N* sodium thiosulphate and the end-point was obtained by using starch solution. Before this procedure was adopted, the inherent errors were calculated and found to give rise to an insignificant error in the determination of the oxygen in the diluted sample.

Modifications to standard procedure

In the initial experiments an occasional release of iodine was observed, presumably caused by local heat generated during neutralisation by the sulphuric acid. The recommended reagent concentrations[3] were diluted (1 + 4) with water and no further error from this source was encountered.

Experience showed that, for oxygen contents from 0·012 to 0·060 p.p.m., 0·005 *N* sodium thiosulphate was suitable for titrating the liberated iodine; for lower oxygen contents, 0·002 *N* sodium thiosulphate was suitable.

Discrepancies up to 0·006 p.p.m. have occasionally been observed and are attributed to poisoning of the platinum electrode used in the potentiometric titration. It is possible to observe when the platinum electrode is failing by taking continuous readings of the e.m.f. after an increment has been added just before the end-point. A rapid drift to a higher e.m.f. is observed, which is markedly different from the normal experience; this variation usually occurs after about 30 titrations. The causes of this behaviour are at present being investigated and, pending the outcome, a new platinum-foil electrode is prepared when abnormal behaviour is noted.

Discussion of results

The results in Table I show that the mean of the differences between the oxygen added and the oxygen measured is nil and the standard deviation of these differences is 0·0018 p.p.m., which compares favourably with the specification[3] of a precision of 0·002 p.p.m. and an accuracy of 0·003 p.p.m. or 1 per cent., whichever is the greater. This is in accordance with the A.S.T.M. definitions of these terms.[9]

As results were primarily intended to be within 0·003 p.p.m. of the true value, it was considered that the possibility of residual oxygen being present in the de-aerated water and reagents could be neglected. The amount of residual oxygen can be determined by making many measurements at "zero" oxygen concentration and is probably similar to that found by Potter (0·0006 p.p.m.).[2]

The proposed apparatus frees the reagents from dissolved oxygen and so overcomes the need to apply a correction of up to 0·010 p.p.m.[3] for this oxygen. This correction must be suspect when less than 0·010 p.p.m. of dissolved oxygen is being determined, as it is affected by temperature and pressure; further, it is unsatisfactory to make a correction of the same order as the amount being measured.

The apparatus is considered to be suitable for the investigation of methods of determining dissolved oxygen, as, irrespective of the method used and the conditions under which the reaction takes place, each result can be independently checked, as it is obtained, by comparing the oxygen measured with that injected. This makes it virtually impossible to reach a wrong conclusion about the accuracy of a particular method.

TABLE I

RECOVERY OF DISSOLVED OXYGEN

Dissolved oxygen injected, p.p.m. × 10^3	Dissolved oxygen found, p.p.m. × 10^3	Difference, p.p.m. × 10^3
0	0	0
0	1	+1
0	1	+1
0	0	0
0	1	+1
0	−3	−3
0	1	+1
0	−1	−1
5	5	0
6	7	+1
6	6	0
6	8	+2
12	14	+2
15	18	+3
17	16	−1
18	17	−1
18	18	0
26	28	+2
27	29	+2
27	26	−1
28	23	−5
28	26	−2
35	38	+3
36	34	−2
36	36	0
37	36	−1
37	37	0
48	48	0
48	49	+1
49	46	−3

The results appear to justify the various novel features incorporated in the apparatus. In particular, it would appear possible to obtain accurate results with this apparatus, although the normal practice of simultaneously preparing a sample and a blank is not followed. The fact that air-saturated water is not added to the blank does not affect the results. This would be expected when only de-mineralised water was used, as the blank is affected not by the oxygen content of the solution but only by interfering substances, none of which should be present in such experiments. Also, the method used to collect the blank probably gives a solution having a high oxygen content and the addition of the small amount of oxygen in the sample would have little effect.

It should be noted that the apparatus is primarily intended for laboratory investigation of methods for determining dissolved oxygen, but, with modifications, it could conceivably be used for the analysis of boiler-feed water.

In order to obtain results in the presence of reducing agents, the proposed procedure has been modified and the blank determined under a nitrogen atmosphere throughout. It is hoped to report on this in due course.

I acknowledge the help received during this work from Dr. C. D. Weir, formerly of this department, and thank Professor J. Small and Mr. W. W. Mackie, of the Mechanical Engineering Department, for their kind interest throughout. I also thank the Faculty of Engineering for financial assistance permitting this work to be carried out.

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The Determination of Traces of Rare Earths in Zirconium and Its Alloys

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A procedure is described for determining rare earths in zirconium and its alloys; it is based on solution of the sample in hydrofluoric acid, addition of yttrium and ytterbium, precipitation of yttrium and rare-earth fluorides, purification of the precipitate by chemical procedures and spectrographic evaluation of each individual rare earth in the final oxide residue. Yttrium is added as a carrier and is also used as an internal standard in the final stages of the procedure. Ytterbium is added as a "control" on the recovery of other rare earths.

The limit of detection for each specified rare earth varies between 0·02 p.p.m. for ytterbium and about 0·5 p.p.m. for cerium. It is estimated that the lower limit of determination for the total rare earths is about 3 p.p.m.

The time taken for a single determination, which includes all the rare earths, is 1½ to 2 days.

BECAUSE of the relatively high thermal neutron absorption cross-section of the rare-earth group of elements, only trace amounts can be tolerated in zirconium-base materials used in thermal reactors.[1] Specifications for reactor-grade zirconium limit the concentration of total rare earths to 15 p.p.m., and this necessitated the provision of a reliable analytical procedure.

Analytical procedures based on spectrographic examination of mixed rare-earth oxides, after a preliminary concentration and purification, are among the most sensitive and accurate methods for determining extremely small amounts of rare earths. Such a procedure has been described by Hettel and Fassel for the determination of fractional parts per million of several rare earths in reactor-grade zirconium.[2]

In Hettel and Fassel's procedure the sample is dissolved in hydrofluoric acid and the solution is passed through a cation-exchange column. This separates zirconium, as the fluorozirconate anion, from yttrium (added as a carrier), rare earths and other cations, which are retained on the column and subsequently eluted and purified by a series of chemical procedures. Gadolinium, terbium, holmium, dysprosium and samarium are determined spectrographically in the final oxide residue.

This procedure takes about 4 days, and a quicker and more suitable method for control purposes was therefore desirable.

In this paper is described the development of such a procedure, in which ammonium fluoride is added to increase the fluoride ion concentration so that the solubility products of the rare-earth fluorides are exceeded.

Experimental

Development of spectrographic procedure for determining rare earths in yttrium oxide—

The complexity of the zirconium spectrum and the comparative weakness of rare-earth spectra make a chemical separation of these constituents an essential prerequisite of any spectrographic procedure for determining rare earths in zirconium. This part of the development work, based on precipitation of rare-earth fluorides with an yttrium carrier, is described later.

The subsequent spectrographic procedure was therefore reduced to determining the concentration of rare earths in what would be essentially an yttrium matrix.

Several methods are available for the spectrographic examination of oxide samples, *e.g.*, high-current d. c. arc,[2] conducting briquette[3] and oxide - resin methods,[4] but, although Hettel and Fassel used the d. c.-arc method, the oxide - resin method was preferred because we had experience of this method in other determinations. In the oxide - resin method the finely powdered oxide is mixed with 1 drop of a thermo-setting resin on the end of a pure graphite rod. After the resin has been cured a powerful discontinuous condenser discharge is passed between this rod and a graphite counter-electrode, and the resulting spectrum is examined.

Several factors were investigated in order to establish optimum conditions for the determination of rare-earth oxides in yttrium oxide. The maximum amount of oxide that could be readily examined with ¼-inch diameter graphite rods was found to be 5 mg. No increase in sensitivity was obtained by using a larger diameter graphite rod, as only the centre portion, about ¼-inch diameter, was sampled by the discharge.

Bakelite Damarda brand resin was suitable and a minimum amount of it was used so that a solid compact layer was formed after curing. A temperature of 130° C and a curing time of 1 hour gave samples of a consistent form.

In order to produce a sufficiently dense spectrum, a high-energy condenser discharge is necessary. Satisfactory spectra were obtained from a B.N.F. "General Purpose Source Unit," with parameters of 250 μF, 10 ohms and 0·06 mH, the sample used as the negative electrode and a gap length of 4 mm. Exposure periods of 30 seconds with the Hilger large-quartz spectrograph provided spectra of adequate density, and because moving-plate exposures showed practically uniform emission characteristics during the first 30 seconds of the discharge, no pre-burn period was used.

Reference to Tables of spectrum lines showed that the majority of the sensitive lines of rare earths lay in the region 2750 to 4700 A, and all subsequent exposures were made with the spectrograph set to this wavelength range.

With a view to determining the most sensitive lines for each rare earth and at the same time obtaining an indication of the limits of detection, a series of samples was prepared, each comprising a mixture of an individual rare-earth and yttrium oxides. The concentrations were equivalent to 1·0 and 10 p.p.m. of rare earth in zirconium, based on the recovery from a 20-g sample of zirconium, in the presence of 20 mg of yttrium oxide carrier. The three most sensitive lines of each rare earth, free from interference by lines of other rare earths, are listed in Table IV. In all instances the limit of detection was less than 1 p.p.m., and further standards were therefore prepared covering the range 0·1 to 2 p.p.m. of each rare earth (based on a 20-g sample and 20 mg of yttrium oxide). Spectra obtained from these standard samples were kept for use as standard comparison spectra for future determinations of rare earths. The limits of detection established were 0·5 p.p.m. for La, Sm, Pr, Nd and Ce and less than 0·1 p.p.m. for Tm, Yb, Er, Eu, Tb, Gd, Dy, Ho and Lu.

The use of internal-comparison line pairs was considered, but was found to be impracticable for a wide concentration range owing to a lack of suitable yttrium lines. It was possible, however, to compile some equality line pairs for use in determining small amounts of rare earths near to the limit of their detection (see Table IV). Use of these equalities permits estimates to be made at the lowest levels without reference to standard spectra, thus reducing the time spent in interpreting spectra when a very small amount of an individual rare earth is present.

The spectrum of a sample of yttrium oxide, with additions of most of the rare earths, was examined and, by comparison with standard spectra, the individual rare-earth contents were determined (see Table I). Reasonable agreement was obtained for each element and the determined total rare-earth content was in excellent agreement with the amount added.

TABLE I

SPECTROGRAPHIC DETERMINATION OF RARE EARTHS IN YTTRIUM OXIDE

Element	Amount added,* as p.p.m. in zirconium	Amount found,* as p.p.m. in zirconium
Thulium	0·2	0·15
Ytterbium	0·4	0·4
Erbium	0·4	0·4
Europium	0·2	0·3
Samarium	0·4	0·7
Terbium	0·2	0·2
Gadolinium	1·0	0·8
Dysprosium	0·2	0·4
Holmium	1·0	1·0
Neodymium	0·4	0·5
Praseodymium	1·0	0·7
Lanthanum	0·4	0·3
Total rare earths	5·8	5·85

* Calculated on the assumption that a 20-g sample of zirconium and 20 mg of yttrium oxide carrier were used.

TABLE II

RESULTS BY THE RECOMMENDED PROCEDURE BASED ON PRECIPITATION OF RARE-EARTH FLUORIDES

Element	Amount added, p.p.m.	Recovered from a solution containing 20 g of— commercially pure zirconium, p.p.m.	Zircaloy 2, p.p.m.	Zr - Cu - Mo alloy, p.p.m.
Lanthanum	0·4	0·3	$0·2_5$	—
Cerium	1·0	1·0	1·0	—
Praseodymium	0·4	0·4	0·4	—
Neodymium	0·4	0·8	1·0	—
Samarium	1·0	1·0	1·0	—
Europium	0·4	0·2	0·2	—
Gadolinium	0·2	0·2	0·3	—
Terbium	0·2	0·2	0·1	—
Dysprosium	0·4	0·4	0·4	—
Holmium	0·2	0·2	0·2	—
Erbium	0·4	0·3	0·3	—
Thulium	0·2	0·3	0·3	—
Ytterbium	0·4	0·5	0·5	—
Total	5·6	5·8	$5·9_5$	
Lanthanum	0·4	0·3	0·3	0·3
Europium	0·4	0·3	0·2	0·3
Dysprosium	0·4	0·3	$0·2_5$	0·3
Ytterbium	0·4	0·3	0·2	0·3
Total	1·6	1·2	$0·9_5$	1·2

SEPARATION OF RARE EARTHS FROM ZIRCONIUM AND THEIR CHEMICAL PURIFICATION—

A 20-g sample of commercially pure zirconium was dissolved in dilute hydrofluoric acid, 20 mg of yttrium oxide (as a solution in hydrochloric acid) were added and the solution was then oxidised with concentrated nitric acid. Yttrium and rare earths were precipitated with ammonium fluoride, the solution was filtered and the precipitate was washed, dried and ignited at 800° C. The precipitate was dissolved in concentrated sulphuric acid and the

solution was evaporated to dryness. The residue was then purified by precipitation of yttrium and rare earths, first as fluorides then as oxalates (as described later). The recovered oxalates were then ignited to oxides and the residue was examined spectrographically, as described previously. No rare earths were detected.

The procedure was repeated with the addition of the equivalent of 0·4 p.p.m. of ytterbium and 20 mg of the yttrium carrier. The amount of ytterbium recovered was equivalent to 0·3 p.p.m. and in repeat tests recoveries of 0·3 and 0·4 p.p.m. were obtained. In each instance the weight of the final residue was about 15 mg. These preliminary tests showed that an acceptable recovery of added ytterbium had been achieved and indicated that similar recoveries of other rare earths could be expected.

The procedure was then applied to solutions containing a 20-g sample of commercially pure zirconium, Zircaloy 2 or zirconium - copper - molybdenum alloy, with rare earth additions, and satisfactory results were obtained (see Table II). These results also indicate that the total rare-earth content of the material examined is well below the specification limit (<15 p.p.m.) for these materials. The time for a complete determination was 1½ to 2 days.

Method

Special reagents—

Yttrium carrier solution—Dissolve 2 g of Specpure yttrium oxide in about 50 ml of dilute hydrochloric acid (1 + 9), and dilute to 1 litre.

Standard rare-earth solutions—Prepare from rare-earth oxides of purity greater than 99·8 per cent. (except europium—purity 95 per cent.). Dissolve, separately, the amounts of rare-earth oxides (except CeO_2) listed in Table III in about 5 ml of hydrochloric acid, sp.gr. 1·18, and dilute each solution to 250 ml. Fuse the CeO_2 with 2 g of potassium hydrogen sulphate in a small platinum dish, extract the cold melt with about 25 ml of dilute hydrochloric acid (1 + 9), and dilute to 250 ml.

Table III

Weight of rare-earth oxide required for standard solution

Oxide	Weight, mg	Oxide	Weight, mg
La_2O_3	11·7	Tb_2O_3	11·5
CeO_2	12·3	Dy_2O_3	11·5
Pr_6O_{11}	12·1	Ho_2O_3	11·4
Nd_2O_3	11·6	Er_2O_3	11·4
Sm_2O_3	11·6	Tm_2O_3	11·4
Eu_2O_3	11·6	Yb_2O_3	11·4
Gd_2O_3	11·5	Lu_2O_3	11·4

Dilute 25 ml of each solution to 250 ml; 1 ml then contains the equivalent of 0·2 p.p.m. of the specified rare earth, based on a 20-g sample.

Preparation of standard spectra—

Place 0·5, 1·0, 2·0, 4·0, 7·0 and 10·0-ml portions of each standard rare-earth solution in separate beakers, and add to each 10 ml of the yttrium carrier solution. Proceed with each of the six solutions as described below—

> Heat to boiling, add 5 ml of ammonium hydroxide, sp.gr. 0·925, and set aside for 15 minutes at 90° C. Cool, filter through a 9-cm Whatman No. 541 filter-paper, and wash the precipitate with dilute ammonium hydroxide (1 + 49). Transfer paper and precipitate to a silica crucible, dry, char, etc., with the usual precautions, and finally heat at 800° C for 15 minutes. Cool, transfer the residue to an agate mortar, and grind. (This entire procedure is necessary for the preparation of each standard rare-earth spectrum.)

Electrodes—

Prepare ¾-inch lengths of ¼-inch diameter graphite rods (Johnson and Matthey type JM4B) with shallow craters cut in one end. Weigh 5 mg of the residue, place 1 drop of Damarda brand resin (Bakelite Ltd.) in the crater on the end of a rod, and transfer the residue to the resin. Mix the residue and resin thoroughly with a small glass rod until a uniform smooth paste is formed. Cure the resin by heating in an oven at 130° C for 1 hour. Several

electrodes, supported in Tufnol blocks, with $\frac{9}{32}$-inch holes drilled in them, may be cured simultaneously.

EXCITATION—

Expose the electrodes spectrographically under the conditions described below; it is convenient to expose each set of standards in order of increasing concentration, a separate plate being used for each rare earth.

Spectrograph—Hilger large quartz.

Wavelength range—2750 to 4700 A.

Plate—Kodak B.10.

Excitation conditions—B.N.F. "General Purpose Source Unit" with parameters 250 μF, 10 ohms and 0·06 mH. Sample used as negative electrode.

Counter-electrode—$\frac{1}{4}$-inch diameter JM4B graphite, 90° cone.

Gap length—4 mm.

Exposure period—No pre-burn; 30 seconds' exposure.

Develop the plate for 2 minutes at 20° ± 0·5° C with Johnson's Universol developer diluted (15 + 85). Rinse, fix, thoroughly wash, and then dry in a dust-free atmosphere.

PROCEDURE FOR SEPARATING AND PURIFYING RARE EARTHS—

A blank determination is usually unnecessary.

Transfer a 20-g sample of zirconium to a 500-ml polythene beaker, add 350 ml of water and then 55 ml of 40 per cent. hydrofluoric acid, in 5-ml portions. When the sample has dissolved, add 2·5 ml of nitric acid, sp.gr. 1·42, and place the beaker in a boiling-water bath until the solution is clear. Cool, and add 10 ml of yttrium carrier solution and 2 ml of ytterbium solution (1 ml ≡ 0·2 p.p.m. of ytterbium, based on a 20-g sample). Add 50 ml of 40 per cent. ammonium fluoride solution and a small amount of paper pulp, mix, and set aside for about 1 hour. Filter through a paper-pulp pad; use polythene apparatus, and wash the precipitate four times with dilute hydrofluoric acid (1 + 40).

Transfer the paper and precipitate to a small platinum dish, dry, char, and finally heat at 800° C for 15 minutes. Cool, add 5 ml of sulphuric acid, sp.gr. 1·84, and heat to dryness. Digest the residue at about 90° C with 25 ml of 40 per cent. hydrofluoric acid for about 15 minutes or until the residue is white. Cool, filter through a Whatman No. 540 filter-paper; use polythene apparatus, and wash the residue with dilute hydrofluoric acid (1 + 40). Transfer the paper to a small platinum dish, and ignite as described previously.

To the cold residue add 5 ml of sulphuric acid, sp.gr. 1·84, and evaporate to dryness. Dissolve the residue in 4 ml of hydrochloric acid, sp.gr. 1·18, dilute to about 25 ml, and transfer the solution to a 150-ml beaker. Dilute to about 100 ml, heat to boiling, and add a boiling solution of 2 g of oxalic acid dissolved in about 25 ml of water. Set the test solution aside overnight, and then filter through a Whatman No. 540 filter-paper, wash with 1 per cent. oxalic acid solution, and ignite, with the usual precautions, in a silica crucible at a final temperature of about 800° C. Cool, transfer the residue to an agate mortar, and grind.

PROCEDURE FOR SPECTROGRAPHIC EXAMINATION OF THE RESIDUE—

Prepare duplicate electrodes, and excite them as described under "Excitation."

Evaluate the spectra by comparing intensities of the lines (see Table IV) in sample and standard spectra, and determine the concentration of the various elements detected (see Note). Compare three lines for each element, and take the mean of the determined values in order to minimise the effect of possible interference. Use internal comparisons whenever possible, *i.e.*, establish an equality or near equality of intensity between rare-earth and nearby yttrium lines, in both sample and standard spectra. For this purpose, the yttrium lines shown in Table IV are satisfactory for the concentrations shown.

NOTE—It is advisable to make a preliminary semi-quantitative examination of the sample spectrum by reference to a spectrum of a specially prepared sample containing detectable amounts of each rare earth. Only elements that are positively identified as being present need then be determined by comparison with standard spectra.

CONCLUSIONS

The recommended procedure based on precipitation and recovery of rare-earth fluorides from zirconium, with subsequent purification and spectrographic determination, is suitable

for determining rare earths in commercially pure zirconium, Zircaloy 2 and zirconium - 0·5 per cent. of copper - 0·5 per cent. of molybdenum alloy.

Table IV

Wavelengths of lines used in the determination of rare earths in zirconium and its alloys

Element		Wavelength, A	Interference, A		Limit of detection,* p.p.m.	Yttrium comparison line, A	Interference, A		Approximately equal at, p.p.m.
Thulium	(1)	2869·2			0·10	2860·7			0·5
	(2)	3133·9	Ni	3134·1	0·10	3133·3			1·2
	(3)	3258·0			0·10	3257·5			1·4
Ytterbium	(1)	3289·4 3289·9			0·01	3278·4			0·3
	(2)	2891·4			0·02	2871·3			0·5
	(3)	3464·4			0·02	3441·0	Fe Zr	3441·0	0·5
Lutecium	(1)	3376·5			0·1	3386·0	Cr	3385·3 3386·5	0·3
	(2)	2911·4			0·1	2882·5			0·3
	(3)	2900·3			0·2	2882·5			0·8
Erbium	(1)	2910·4	Zr	2910·2	0·10	2882·5			0·2
	(2)	2904·5			0·10	2902·7	Mo	2903·1	0·2
	(3)	3264·8			0·10	3257·5			0·2
Europium	(1)	2906·7			0·10	2902·7 2882·5			0·1 0·5
	(2)	3972·0	Zr	3972·3	0·10	3973·5			0·5
	(3)	3907·1			0·10	3924·0			0·3
Samarium	(1)	4280·8	Ca	4283·0	0·50	4277·5	Zr	4277·4	0·8
	(2)	3321·2			0·50	3320·5			1·4
	(3)	3670·8			0·50	3671·3	Zr	3671·3	1·0
Terbium	(1)	3324·4			0·10	3316·5			0·5
	(2)	4326·5	Fe	4325·8	0·10	4320·0			0·2
	(3)	3976·8			0·40	3978·0	Zr	3977·5	1·0
Gadolinium	(1)	3422·5			0·05	3421·0			0·1
	(2)	3350·5			0·05				
	(3)	3646·2			0·10	3650·0			0·5
Dysprosium	(1)	3645·4			0·10	3650·0			0·1
	(2)	3385·0			0·20	3386·0	Cr	3385·3 3386·5	0·5
	(3)	3393·6			0·20	3393·0	Zr	3393·1	1·0
Holmium	(1)	3399·0	Zr Fe	3399·3	0·10	3406·0			0·4
	(2)	3416·5			0·10	3406·0			0·6
	(3)	3453·1	Ni	3452·9	0·10	3452·5			0·5
Neodymium	(1)	4012·3	Ce Zr	4012·3 4012·3	0·40	3997·8			1·0
	(2)	3963·1	Ti	3962·9	0·80	3978·0	Zr	3977·5	1·0
	(3)	3990·1	Ti	3990·2	0·80	3996·5			1·0
Praseodymium	(1)	4008·7	Er	4008·2	0·50	4011·0			1·0
	(2)	3964·8 3965·3 3966·6			0·30	3978·0	Zr	3977·5	1·0
	(3)	4468·7	Zr	4468·8	1·0	4463·5			2·0
Lanthanum	(1)	4333·7	Zr	4333·3	0·2	4325·0	Fe	4325·8	1·0
	(2)	3995·8			0·2	3997·8			1·2
	(3)	4031·7			0·2	3997·8			1·5
Cerium	(1)	4012·3	Nd Zr	4012·3 4012·3	0·5	4011·8			0·5

* Limits of detection given above assume no interference from other elements.

The lower limit of detection of the spectrographic procedure for individual rare earths varies between 0·02 p.p.m. for ytterbium and 0·5 p.p.m. for cerium; the lower limit for total rare earths is about 3 p.p.m. If required, the sensitivity of the method can be increased by using a larger weight of sample.

A single sample can be analysed in 1½ to 2 days, and experience has shown that about six samples can be analysed in 3 to 4 days.

By the recommended procedure the rare-earth content of typical zirconium-bearing materials has been shown not to exceed about 5 p.p.m., which is well within a specification limit of 15 p.p.m.

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Determination of the Permanganate Value for Waters and Sewage Effluents Containing Nitrite

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The results of a comparative study of the efficiencies of urea and sulphamic acid in the destruction of nitrites added to distilled water and sea water, or present in sewage effluents, before the determination of permanganate values are presented and discussed. Sulphamic acid is preferred for reasons of efficiency, economy and suitability, especially when phosphoric acid is used to acidify the samples.

One of the earliest tests for assessing the quality of sewage effluents and the pollution of water was the determination of the oxygen absorbed from acidified potassium permanganate solution. The development of this test in 1850, its continued use and its limitations have been recently reviewed.[1,2] A point of considerable interest about the "permanganate value,"[2] apart from its determination being simple and fairly rapid, is that it provides a continuous basis for comparison with the results accumulated over many years. G. J. Fowler, in unpublished work, observed that for many domestic sewages a 3-minute oxygen absorption of 3·6 p.p.m. indicated satisfactory purification and that for Manchester sewage, which contained much oxidisable industrial refuse, 7·1 p.p.m. might be accepted. The determination of the oxygen absorbed from permanganate in 3 minutes, *i.e.*, practically instantaneously, gives a means of differentiating between readily or easily oxidisable substances and more slowly or difficultly oxidisable substances, which are determined by the 4-hour test.

Since soon after the adoption of the test, it has been known that the presence of nitrites in the samples interferes with the determination of the permanganate value.[3] Quantitative determination of the nitrite and calculation of its effect on the permanganate demand, the result being recorded as "permanganate value corrected for nitrite," is a somewhat tedious procedure, and it is preferable to destroy the nitrite. Jung and Otto[4] tried to overcome the effect of nitrite by adding sodium azide, but found that an excess of azide affected the permanganate demand; they recommended the addition of 50 mg of urea to a water containing 100 mg of nitrite per litre Nolte and Bandt[5] found that the destruction of nitrite by urea was slow in the cold, but could be accelerated by gently warming the mixture. Two years ago a Joint Committee of the Association of British Chemical Manufacturers and the Society for Analytical Chemistry[2] recommended that 1 g of urea be added to the acidified sample containing nitrite and to the blank and that the solutions be set aside for 5 minutes before the appropriate volume of permanganate solution is added.

In this connection we have found that sulphamic acid is more efficient than urea and that its use is more economical, especially in the analysis of saline and sea waters containing nitrite. The results of a comparative study of the use of sulphamic acid and urea in the determination of permanganate values are described in this paper. Sulphamic acid has been used in a modification of the Winkler method for determining dissolved oxygen[6,7,8]; its application in the oxygen-absorption test does not seem to have been attempted.

Materials and Methods

Samples of distilled water and sea water to which different amounts of sodium nitrite had been added and samples of sewage effluents containing different amounts of nitrite were used for the oxygen-absorption tests. Reagents were prepared and tests were carried out as recommended by the Joint Committee.[2]

Results

Eight series of tests were carried out to study the relative efficiencies of urea and sulphamic acid for destroying nitrite in water samples and sewage effluents. In the first series, 100-ml samples of distilled water containing 1 or 2 mg of added nitrite-N were treated with 1-ml portions of 0·5 *M* solutions of urea or sulphamic acid, *i.e.*, 30 mg of urea or 48·5 mg of sulphamic acid. The 3-minute permanganate values were determined after the solutions had been set aside for periods of up to 5 minutes. (The permanganate values of 100-ml samples containing 1 and 2 mg of nitrite-N were 11·2 and 22·5 p.p.m., respectively.) For the urea solution, the results were—

Time after addition of urea solution, minutes	1	3	5
Permanganate value in presence of 1 mg of nitrite-N, p.p.m. ..	9·8	9·0	8·2
Permanganate value in presence of 2 mg of nitrite-N, p.p.m. ..	20·2	18·3	16·9

When 0·5 *M* sulphamic acid was used, the 3-minute permanganate value was 0·0 p.p.m. in each test.

The rate of destruction of nitrite-N in 100-ml samples of water by 1-ml portions of 0·5 *M* urea solution was then determined; the results were—

Time after addition of urea solution, minutes	1	3	5	10	15	20	25	30	45	60
Permanganate value in presence of 1 mg of nitrite-N, p.p.m.	9·7	9·0	8·0	6·2	4·7	3·4	2·9	2·2	1·4	0·4
Permanganate value in presence of 2 mg of nitrite-N, p.p.m.	20·2	18·2	16·4	12·9	10·2	8·6	6·9	5·2	3·2	1·6

The extent to which nitrite was destroyed was determined by adding increasing amounts of urea or the theoretical amounts of sulphamic acid to 100-ml samples of water containing known amounts of added nitrite-N; the results are shown in Table I. When twice the theoretical amount of sulphamic acid was added, destruction of nitrite was almost instantaneous.

The efficiency of sulphamic acid for destroying nitrite was then studied. Different amounts of nitrite were added to 100-ml samples of distilled water and the 3-minute and 4-hour permanganate values were determined. The results were—

Amount of nitrite-N added, mg	0·2	0·4	0·6	0·8	1·0	2·0	3·0	4·0	5·0
3-minute permanganate value, p.p.m. ..	2·2	4·4	6·6	9·0	11·2	22·5	34·0	44·9	55·6
4-hour permanganate value, p.p.m. ..	2·2	4·6	6·6	9·0	11·4	22·9	34·2	45·3	56·2

The series was then repeated, 1 ml of 0·5 *M* sulphamic acid being added 1 minute before the permanganate solution; in no test was either the 3-minute or the 4-hour permanganate value greater than 0·1 p.p.m. As the concentration of nitrite-N in polluted waters and sewage effluents does not normally exceed 50 p.p.m. (5 mg per 100 ml), it is suggested that 50 mg of sulphamic acid be added to 100 ml of sample containing up to 5 mg of nitrite-N and to the blank and that the mixture be set aside for 1 minute to ensure complete destruction of the nitrite.

The efficiencies of urea and sulphamic acid for destroying nitrite in sewage effluent were also determined; the results are shown in Table II.

TABLE I

DESTRUCTION OF NITRITE BY UREA AND SULPHAMIC ACID

The 3-minute permanganate values for water samples containing 2 and 5 mg of nitrite-N were 22·4 and 55·8 p.p.m., respectively

Amount of nitrite-N present in sample, mg per 100 ml	Amount of reagent added to 100 ml of sample, mg	3-minute permanganate value after— 1 minute, p.p.m.	2 minutes, p.p.m.	3 minutes, p.p.m.	5 minutes, p.p.m.	10 minutes, p.p.m.
Urea added—						
2	30	20·4	—	18·5	16·6	12·5
	60	18·8	—	14·9	12·9	9·2
	120	16·6	—	11·5	8·1	3·7
	240	12·7	—	6·0	3·3	0·6
	600	4·0	—	0·4	0·1	0·0
5	500	20·8	—	4·4	1·2	0·0
	600	16·4	—	2·4	0·4	0·0
	800	11·2	—	1·2	0·0	0·0
	1000	7·6	—	0·4	0·0	0·0
Sulphamic acid added—						
1	6·93	0·2	0·0	—	—	—
2	13·86	0·2	0·0	—	—	—
3	20·79	0·4	0·0	—	—	—
4	27·72	0·6	0·1	—	—	—
5	34·65	0·6	0·2	—	—	—

TABLE II

EFFICIENCIES OF UREA AND SULPHAMIC ACID FOR DESTROYING NITRITE IN SEWAGE EFFLUENTS

Sulphamic acid or urea was added 2 minutes before the permanganate solution. A 100-ml sample of effluent was used in each test

Amount of nitrite-N present, mg	Calculated permanganate value (A),* p.p.m.	Permanganate value in absence of urea or sulphamic acid (B), p.p.m.	Permanganate value expected ($B - A$), p.p.m.	Permanganate value in presence of 50 mg of sulphamic acid, p.p.m.	Permanganate value in presence of— 50 mg of urea, p.p.m.	300 mg of urea, p.p.m.	1 g of urea, p.p.m.
Three-minute oxygen-absorption test—							
0·0	0·0	2·4	2·40	2·4	2·4	2·4	2·4
0·68	7·75	11·2	3·45	3·6	10·2	6·3	3·6
1·04	11·86	14·6	2·74	2·8	12·2	6·9	2·8
1·32	15·05	18·7	3·65	3·8	15·6	9·7	3·8
1·58	18·01	21·6	3·59	3·6	18·4	10·8	3·6
2·40	27·36	30·6	3·24	3·4	24·9	13·7	4·4
3·24	36·94	40·9	3·96	4·2	33·2	15·9	5·4
Four-hour oxygen-absorption test—							
0·0	0·0	8·8	8·8	8·8	8·8	8·8	8·8
0·68	7·75	17·3	9·55	9·6	15·9	12·3	9·6
1·04	11·86	20·7	8·84	8·8	18·1	12·7	8·8
1·32	15·05	24·8	9·75	9·8	21·7	15·9	9·8
1·58	18·01	27·8	9·79	9·8	24·8	17·0	10·0
2·40	27·36	36·8	9·44	9·6	31·2	19·8	9·8
3·24	36·94	46·9	9·96	10·2	39·3	22·3	10·8

* Amount of nitrite present, mg × 11·4.

As phosphoric acid is used to acidify samples of sea water,[9] the effect of this acid was studied. Different amounts of nitrite were added to 100-ml samples of sea water that had been acidified with phosphoric acid, and sulphamic acid or urea was added 2 minutes before the permanganate solution; the results are shown in Table III.

TABLE III

EFFECT OF PHOSPHORIC ACID IN ANALYSIS OF SEA WATER

Amount of nitrite-N present, mg	3-minute permanganate value— in absence of sulphamic acid or urea, p.p.m.	in presence of 300 mg of urea, p.p.m.	in presence of 50 mg of sulphamic acid, p.p.m.
0	0·2	0·2	
1	11·4	9·3	
2	22·7	17·8	0·2
3	34·2	26·0	
4	45·1	32·3	
5	55·8	45·4	

Finally, the effects of phosphoric acid and sodium chloride on urea were investigated, the results being shown in Table IV. Known amounts of nitrite were added to 100-ml samples of distilled water and the 3-minute permanganate values were determined after the samples had been acidified with (*a*) sulphuric acid and (*b*) phosphoric acid; 300 mg of urea were added to each sample 2 minutes before the permanganate solution. The experiment was then repeated with samples containing added sodium chloride and acidified with phosphoric acid. The 3-minute permanganate values for 100-ml samples of sea water treated with 1 g of urea and acidified with phosphoric acid and containing 5 mg of nitrite-N, after being set aside for 2, 3, 5 and 10 minutes before permanganate solution was added, were 15·0, 7·6, 1·8 and 0·0 p.p.m., respectively.

TABLE IV

EFFECTS OF PHOSPHORIC ACID AND SODIUM CHLORIDE ON UREA

Amount of nitrite-N present, mg	3-minute permanganate value— in absence of urea, p.p.m.	in presence of 300 mg of urea, p.p.m.	in presence of 300 mg of urea and 3 g of sodium chloride,* p.p.m.
Samples acidified with sulphuric acid—			
1	11·2	3·5	—
2	22·5	7·2	—
3	34·0	9·8	—
4	44·9	14·7	—
5	55·6	17·6	—
Samples acidified with phosphoric acid—			
1	11·0	9·1	8·3
2	22·4	17·6	16·8
3	34·0	25·8	26·2
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5	55·4	45·2	45·4

* Similar results were obtained in presence of 1 and 2-g amounts of sodium chloride.

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The results indicate that it is preferable to use sulphamic acid for destroying nitrite in waters and sewage effluents in the determination of permanganate values. In comparison with urea, a much smaller amount of sulphamic acid is needed and destruction of nitrite is more rapid, the reaction of urea being even slower when the samples are acidified with phosphoric acid.

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The cost of sulphamic acid is higher than that of urea, but the greater efficiency of sulphamic acid and the smaller amounts of it needed make it cheaper than urea for this work. These considerations of efficiency and economy become still more important when applied to sea water and waters containing large amounts of sodium chloride.

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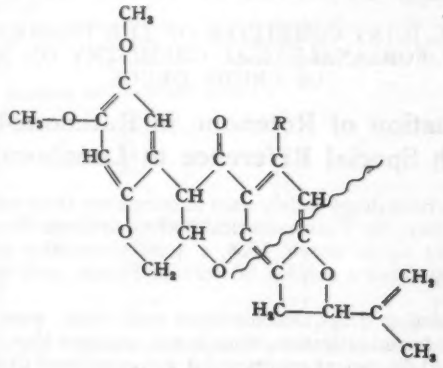

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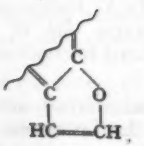

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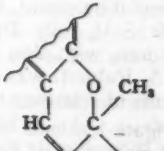

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These compounds are found in many leguminous plants, particularly in the sub-family *Papilionaceae*. The important genera in this sub-family are *Derris*, *Lonchocarpus*, *Tephrosia* and *Milletia*, of which only the first two have become important in commerce. A thriving derris-plantation industry was developed in Malaya, Java and Sumatra, but this suffered from the effects of the second World War. Lonchocarpus, on the other hand, occurs principally in Central and South America. This geographical situation led to its extensive use during that war, and these areas are now the principal source of rotenone-containing materials.

Rotenone and its related compounds occur chiefly in the root of the plant; the principal article of commerce is the dried root, either whole, chopped or, more usually, in powdered form. From the root an extract or resin may be prepared by solvent treatment.

REVIEW OF ANALYTICAL METHODS—

Once the commercial use of derris and lonchocarpus had become firmly established, the determination of rotenone became important. The first quantitative method was proposed by Tattersfield and Roach,[6] who extracted dried and powdered derris root with ether and then weighed the rotenone, which crystallised from a concentrated ethereal solution. This method was superseded by one suggested by Jones,[7] who found that crystallisation from carbon tetrachloride gave more reproducible results and also permitted more rotenone, by weight, to be crystallised out. Rotenone separated from such solutions as a solvate containing one molecule of the solvent to one molecule of rotenone.

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Jones and Graham[9] developed a method on these lines, which was adopted as official by the Association of Official Agricultural Chemists; a variation of this method was proposed by Coomber, Martin and Harper[10] as the standard method in this country, included in the British Pharmaceutical Codex, 1949,[11] and subsequently transferred to the British Veterinary Codex, 1953.[12] A variety of other methods was proposed between 1930 and 1939, but none was so popularly accepted as the carbon tetrachloride method.

The two methods in general use during the first decade after the second World War were those described in the British Veterinary Codex, 1953, and in the Association of Official Agricultural Chemists' Methods of Analysis, 1955.[13]

The method described in the Codex consists in percolation of the ground root with hot chloroform in a continuous-extraction apparatus. An aliquot of the chloroform solution is evaporated and the residue is dissolved in carbon tetrachloride previously saturated with rotenone at 0° C. This solution is cooled in ice for not less than 16 hours, and the separated crystals are collected on a sintered-glass Gooch crucible. The crystals of rotenone - carbon tetrachloride solvate are washed with ice-cold rotenone-saturated carbon tetrachloride and dried at 40° C. The outlet of the Gooch crucible is closed, and the crystals are treated with alcohol previously saturated with rotenone at 0° C. After being set aside at 0° C for not less than 4 hours, the liquid is removed by suction, and the crystals are washed with ice-cold rotenone-saturated alcohol. The residue is dried to constant weight at 105° C, and this weight is noted. A 4 per cent. solution of the residue in benzene is prepared, and its optical rotation at 20° C is determined. This determination permits a calculation of the purity of the rotenone thus separated, and, by appropriate calculations, the rotenone content of the root is established.

TABLE III

EFFECT OF PHOSPHORIC ACID IN ANALYSIS OF SEA WATER

Amount of nitrite-N present, mg	3-minute permanganate value— in absence of sulphamic acid or urea, p.p.m.	in presence of 300 mg of urea, p.p.m.	in presence of 50 mg of sulphamic acid, p.p.m.
0	0·2	0·2	
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2	22·7	17·8	0·2
3	34·2	26·0	
4	45·1	32·3	
5	55·8	45·4	

Finally, the effects of phosphoric acid and sodium chloride on urea were investigated, the results being shown in Table IV. Known amounts of nitrite were added to 100-ml samples of distilled water and the 3-minute permanganate values were determined after the samples had been acidified with (*a*) sulphuric acid and (*b*) phosphoric acid; 300 mg of urea were added to each sample 2 minutes before the permanganate solution. The experiment was then repeated with samples containing added sodium chloride and acidified with phosphoric acid. The 3-minute permanganate values for 100-ml samples of sea water treated with 1 g of urea and acidified with phosphoric acid and containing 5 mg of nitrite-N, after being set aside for 2, 3, 5 and 10 minutes before permanganate solution was added, were 15·0, 7·6, 1·8 and 0·0 p.p.m., respectively.

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EFFECTS OF PHOSPHORIC ACID AND SODIUM CHLORIDE ON UREA

Amount of nitrite-N present, mg	3-minute permanganate value— in absence of urea, p.p.m.	in presence of 300 mg of urea, p.p.m.	in presence of 300 mg of urea and 3 g of sodium chloride,* p.p.m.
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1	11·2	3·5	—
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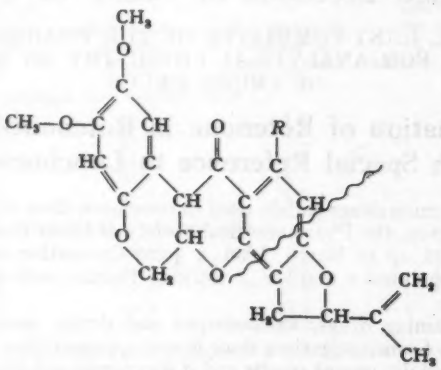

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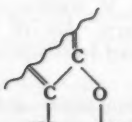

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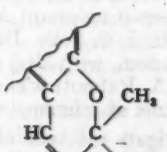

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Cahn and Boam[8] also worked on a method involving crystallisation from carbon tetrachloride and noted that some rotenone remained behind in the mother liquor, particularly when the rotenone content of the extract was low. They showed that crystallisation was facilitated by the addition of a weighed amount of rotenone. They also considered that, if the amount of crude rotenone obtained were taken as the true rotenone content, then losses in the mother liquor were probably compensated for by the low purity of the crystals that separated.

Jones and Graham[9] developed a method on these lines, which was adopted as official by the Association of Official Agricultural Chemists; a variation of this method was proposed by Coomber, Martin and Harper[10] as the standard method in this country, included in the British Pharmaceutical Codex, 1949,[11] and subsequently transferred to the British Veterinary Codex, 1953.[12] A variety of other methods was proposed between 1930 and 1939, but none was so popularly accepted as the carbon tetrachloride method.

The two methods in general use during the first decade after the second World War were those described in the British Veterinary Codex, 1953, and in the Association of Official Agricultural Chemists' Methods of Analysis, 1955.[13]

The method described in the Codex consists in percolation of the ground root with hot chloroform in a continuous-extraction apparatus. An aliquot of the chloroform solution is evaporated and the residue is dissolved in carbon tetrachloride previously saturated with rotenone at 0° C. This solution is cooled in ice for not less than 16 hours, and the separated crystals are collected on a sintered-glass Gooch crucible. The crystals of rotenone - carbon tetrachloride solvate are washed with ice-cold rotenone-saturated carbon tetrachloride and dried at 40° C. The outlet of the Gooch crucible is closed, and the crystals are treated with alcohol previously saturated with rotenone at 0° C. After being set aside at 0° C for not less than 4 hours, the liquid is removed by suction, and the crystals are washed with ice-cold rotenone-saturated alcohol. The residue is dried to constant weight at 105° C, and this weight is noted. A 4 per cent. solution of the residue in benzene is prepared, and its optical rotation at 20° C is determined. This determination permits a calculation of the purity of the rotenone thus separated, and, by appropriate calculations, the rotenone content of the root is established.

The method recommended by the Association of Official Agricultural Chemists differs slightly in that the ground root is extracted with chloroform at room temperature by placing the flask containing the ground root and solvent on a shaking machine for several hours. After filtration, an aliquot of the chloroform solution is evaporated. The residue is dissolved in carbon tetrachloride, and the whole is kept in a bath of ice for 17 to 18 hours; the separated crystals are then filtered and washed with ice-cold carbon tetrachloride previously saturated with rotenone at 0° C. The separated crystals are dried to constant weight at 40° C, and this weight is recorded. The dried material is then removed from the filter, and a 1-g portion is treated with alcohol previously saturated with rotenone at room temperature. After mixing, the whole is set aside for at least 4 hours, and the crystals are then separated on a Gooch crucible and washed with alcohol saturated with rotenone at room temperature. The residue is dried to constant weight at 105° C. Multiplication of this weight of residue by the total weight of the crude rotenone - carbon tetrachloride solvate gives the weight of pure rotenone contained in the aliquot taken. A correction to this weight is made for the rotenone held in solution in the carbon tetrachloride used in the crystallisation.

After 1945, it soon became apparent that the existing methods gave widely divergent results in different laboratories. When reviewing these methods, the attention of the Panel was drawn to work published during the war by Martin[14] wherein the earlier work of Tattersfield and Martin[15] was further developed. Tattersfield and Martin had shown that the phenolic and emulsifying constituents of derris extract could be removed by washing with alkali; once these materials were removed, it was found that the crystallisation of rotenone from carbon tetrachloride was greatly facilitated. The results of Martin's researches had escaped attention and were not utilised in analytical procedures when the use of derris and lonchocarpus was revived.

Experimental

Analysis of lonchocarpus resin—

Collaborative tests were first carried out on a sample of ground lonchocarpus resin. Most of the collaborators normally used their own variants of the method recommended by the Association of Official Agricultural Chemists in their own laboratories; therefore, in order to determine the merit of each worker's own method, the sample was assayed both by the method described in the British Veterinary Codex, 1953, which was strictly followed, and by the worker's own method. The results are shown in Table I.

Table I

Analysis of lonchocarpus resin by different methods

Laboratory	Rotenone found by— method described in British Veterinary Codex, %	Rotenone found by— worker's own method, %
A	36·8, 36·7, 36·7	37·5, 38·1, 37·2
B	28·9, 30·4, 30·3	35·0, 34·0, 34·6
C	31·3, 31·7, 31·7	29·4, 29·9, 28·5
D	31·7, 31·3, 32·2	31·0, 31·3, 33·3
E	29·3, 28·2, 27·9	Uses B.Vet.C. method
F	32·2, 33·7, 32·1	34·3, 34·5, 34·2
G	32·9, 33·1, 33·2	37·6, 36·5, 36·3
H	32·4, 33·3, 33·8, 32·8	34·8, 35·8, 34·8, 34·1
Mean	32·2	34·2
Standard deviation ..	2·31	2·65
Coefficient of variation ..	7·17	7·75

It was desirable that the over-all variation in the results by the British Veterinary Codex method should be reduced, but detailed examination of the workers' own methods failed to reveal any fundamental procedure that could be used as a basis for unification.

Attention was then turned to the work of Martin,[14] in which the solution of the resin was washed with caustic alkali before separating the rotenone - carbon tetrachloride complex by crystallisation. This procedure removed resinous material that interfered with crystallisation of the solvate and had the added advantage of yielding a markedly purer product;

the degree of purity could be determined directly by polarimetry, thereby avoiding treatment with alcohol. A preliminary investigation by the Panel showed that a higher and more consistent figure for rotenone content was obtained by omitting the treatment with alcohol.

It was possible that washing with caustic alkali might cause degradation, and this was investigated by substituting ammonium hydroxide for potassium hydroxide. Most workers reported a greater tendency to emulsification, and results were lower than when potassium hydroxide was used. It therefore appeared that potassium hydroxide was more efficient in extracting the interfering substances, and members of the Panel expressed a preference for its use.

The accuracy of the polarimetric procedure for determining the purity of the solvate was also examined. Concurrently with the work of the Panel, a committee of the Association of Official Agricultural Chemists was conducting a collaborative investigation into the analysis of rotenone-containing material, using a modification of the spectrophotometric procedure proposed by Payfer.[16] The Panel had been apprised of this and accordingly investigated its applicability in determining the purity of the solvate. When this method was used, each worker reported a value differing from that obtained by the optical-rotation method, but there was no constant factor governing the deviation. It was therefore agreed to continue the use of the polarimetric method for determining the purity of the solvate, as this gave more consistent results.

As a result of these various investigations, a procedure was adopted by the Panel. The results of a collaborative trial (with two samples) of the method finally recommended for the analysis of lonchocarpus resin (see Appendix) are shown in Table II.

TABLE II

ANALYSIS OF LONCHOCARPUS RESIN BY THE RECOMMENDED METHOD

Laboratory	Rotenone found in— sample A, %	sample B, %
A	36·2, 35·9, 35·8	—
B	35·6, 35·2, 34·7	44·2, 44·9, 44·0
C	34·7, 34·7, 34·7	43·3, 40·7, 42·1
D	35·6, 36·4, 36·2	42·0, 42·7, 42·7
E	35·7, 37·8, 37·2	43·6, 44·5, 44·6
F	37·4, 38·1, 36·8	44·7, 44·9, 44·8
G	35·8, 35·2, 35·6	42·0, 41·8, 41·3
H	37·7, 37·8, 37·7	42·5, 42·2
Mean	36·2	43·2
Standard deviation ..	1·09	1·30
Coefficient of variation ..	3·01	3·01

ANALYSIS OF LONCHOCARPUS ROOT—

The Panel then directed its attention towards application of the method to the determination of the rotenone content of ground lonchocarpus root. The method recommended by the Association of Official Agricultural Chemists, namely, cold extraction with chloroform (but omitting the use of decolorising charcoal), was used for extracting the active ingredients from the root. The possibility of using the chloroform solution throughout the alkali-washing stage was investigated, but all workers reported troublesome emulsions, and so the chloroform solution was first evaporated to dryness; the residue was dissolved in a mixture of equal parts of benzene and ether, the mixture used in the recommended method.

After several modifications of the technique had been made, in connection with various precautions to be taken in the filtration and evaporation stages, the results were satisfactory. The results of a collaborative trial (with two samples) of the method finally recommended for the analysis of lonchocarpus root (see Appendix) are shown in Table III.

ASSAYS BY INDEPENDENT ANALYSTS—

The same samples of ground lonchocarpus root were assayed by the recommended method by four independent analysts (in the United Kingdom, the U.S.A. and Denmark)

who had no previous experience of the method or communication with members of the Panel. The results are shown in Table IV.

TABLE III

ANALYSIS OF GROUND LONCHOCARPUS ROOT BY THE RECOMMENDED METHOD

Laboratory	Rotenone found in— sample C, %	sample D, %
A	5·99, 6·16, 5·85	5·23, 5·24, 5·27
B	—	5·29, 5·33, 5·42,
C	5·20, 5·21, 5·30	5·89, 5·72, 5·56
D	5·37, 5·48, 5·40	5·49, 5·49, 5·38
E	5·62, 5·65, 5·67	5·76, 5·97, 5·77
F	5·61, 5·38, 5·45	5·59, 5·41, 5·57
G	5·42, 5·53, 5·51	5·86, 5·78, 5·82
H	5·67, 5·82, 5·68	—
Mean	5·57	5·56
Standard deviation ..	0·239	0·229
Coefficient of variation ..	4·29	4·12

TABLE IV

ANALYSIS OF GROUND LONCHOCARPUS ROOT BY INDEPENDENT ANALYSTS

Laboratory	Rotenone found in— sample C, %	sample D, %
J	5·45, 5·45, 5·45	5·35, 5·28, 5·39
K	5·38, 5·33, 5·25	5·52, 5·42, 5·11
L	5·30, 5·18, 5·23	5·20, 5·22, 5·25
M	5.45, 5·61, 5·63	5·05, 5·04, 5·26
Mean	5·39	5·26
Standard deviation ..	0·136	0·141
Coefficient of variation ..	2·52	2·68

CONCLUSIONS

Methods for the assay of lonchocarpus resin and ground root have been tested collaboratively and are recommended. Coefficients of variation of 3·0 for the resin and 4·2 for the ground root indicate an appreciable advance over previously accepted procedures and show that the scatter has been considerably reduced. Results from outside collaborators, with a coefficient of variation of only 2·6 for the ground root, are exceptionally encouraging.

The results obtained are considered to show the method to be satisfactory for the purpose of commercial transactions, provided that strict adherence to precedural detail is observed.

The Panel wish to make acknowledgement to Bugges Insecticides Ltd., The Murphy Chemical Company Ltd., Foster D. Snell Inc., New York, and Dr. P. Terp, Danmark Apotekerforening's Kontrollaboratorium, for their participation in the analyses.

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Appendix

RECOMMENDED METHODS FOR THE DETERMINATION OF ROTENONE

PRINCIPLE OF METHOD—

The resin, or dried root extract, is purified with caustic alkali. The rotenone is extracted and crystallised from carbon tetrachloride. The percentage purity of a solution of the crystals is determined by polarimetry and thence the percentage of rotenone in the sample is calculated.

APPLICABILITY—

For the determination of rotenone in ground lonchocarpus and derris roots and in extractives therefrom.

LONCHOCARPUS RESIN

REAGENTS—

Hydrochloric acid, dilute (*approximately* N).

Potassium hydroxide solution, 2 per cent. w/v.

Potassium hydroxide solution, 5 per cent. w/v.

Benzene - ether solution (1 + 1 *v/v*).

Rotenone—Use material prepared according to the instructions given in the British Veterinary Codex, 1953, p. 626.

Carbon tetrachloride—Analytical-reagent grade.

Carbon tetrachloride, saturated with pure rotenone at 0° *C*—Dissolve 2·72 g of rotenone in 1 litre of carbon tetrachloride, and cool the solution to 0° C.

Benzene—Analytical-reagent grade.

Sodium sulphate, anhydrous, powdered.

PROCEDURE—

Accurately weigh about 5 g of the resin (*W*), dissolve it in 100 ml of benzene - ether solution, and transfer to a pear-shaped 500-ml separating funnel with a further 20 ml of solvent.

Cautiously add 50 ml of 2 per cent. potassium hydroxide solution, allowing it to run down the sides of the separating funnel, carefully turn the separating funnel to a horizontal position, gently rotate it about six times, taking care to avoid emulsification, and carefully return the funnel to the vertical position.

Remove the alkaline layer as rapidly as possible by running it into a second separating funnel containing 40 ml of benzene - ether solution. In order to carry out this separation rapidly, it may be necessary to run off only the clear aqueous portion, leaving in the separating funnel any trace of emulsion. The tendency to emulsify is markedly less in the later washings.

Carry out a second rapid extraction with 50 ml of 5 per cent. potassium hydroxide solution; use an increased degree of agitation, but still take care to avoid emulsification. Repeat the extraction with vigorous shaking, using a further 50 ml of 5 per cent. potassium hydroxide solution, and run each alkaline layer into the second separating funnel. Immediately after the third extraction, add 50 ml of distilled water to the main benzene - ether solution, and shake gently to reduce the concentration of any residual potassium hydroxide.

The whole extraction procedure should not take more than 30 minutes.

After agitation, run off and discard the 150 ml of alkaline extract from the second separating funnel, and add the 40 ml of benzene - ether solution to the main benzene - ether solution in the first separating funnel.

To the 160 ml of benzene - ether solution and 50 ml of water in the first separating funnel add a small piece of litmus paper and then hydrochloric acid, drop by drop, with shaking after each addition, until the paper just turns red. Run off the aqueous layer, and wash the benzene - ether solution with 3 portions, each of 25 ml, of distilled water, allowing effective drainage after each washing. Run off the water, and run the benzene - ether solution through a layer of anhydrous sodium sulphate (about 10 to 15 g) supported in a filter funnel on a plug of cotton-wool. Wash the separating funnel and then the sodium sulphate with 25 to 30 ml of solvent. Remove the solvent by distillation, using a 100-ml flask, on a water bath and then by warming under reduced pressure. Re-dissolve the residue in 15 ml of hot carbon tetrachloride, and evaporate to dryness under reduced pressure as before. Repeat this operation, the final evaporation to dryness under reduced pressure being for about 10 minutes.

Dissolve the residue in 25 ml of carbon tetrachloride saturated with pure rotenone at 0° C by boiling under reflux on a water bath until solution is complete. Swirl the flask in a bath containing a mixture of ice and water until crystallisation appears to be complete. Leave the flask in the bath of ice and water in a refrigerator overnight.

Filter the solution through a tall glass crucible having a sintered-glass filter (porosity No. 3) previously cooled to 0° C, and rapidly wash the crystals with 15 ml (in 3 approximately equal portions) of ice-cold carbon tetrachloride saturated with pure rotenone at 0° C. Maintain suction for about 5 minutes, dry the residue at 40° C for 1 hour, and weigh the dry complex (W_1).

Remove the crystals from the crucible, mix well, and prepare a 4 per cent. w/v solution in benzene. Measure the optical rotation at 20° C in a 10-cm tube.

The specific rotation of a 4 per cent. w/v solution of the pure complex in benzene is −166°. The percentage purity of the complex is therefore given by the expression $100\alpha/6{\cdot}64$, where α is the observed rotation in circular degrees of a 4 per cent. solution at 20° C in a 10-cm polarimetric tube.

CALCULATION—

It has been established by Jones[7] that the solvate formed when rotenone is crystallised from carbon tetrachloride is equimolecular. The percentage of rotenone in the dried solvate is therefore 72. If W is the weight of resin taken, W_1 is the weight of solvate and α is the observed rotation, in circular degrees, of a 4 per cent. solution of the solvate in benzene at 20° C, then the percentage of rotenone can be calculated from the expression—

$$\text{Rotenone content, \%} = \frac{0{\cdot}72 \times W_1}{W} \times \frac{100\alpha}{6{\cdot}64} = \frac{10{\cdot}84\, W_1\, \alpha}{W}$$

GROUND LONCHOCARPUS ROOT

REAGENTS—

As for lonchocarpus resin, together with—

Chloroform, B.P.

PROCEDURE—

Place 30 g of the well mixed sample, ground to pass through a 30-mesh sieve, in a 500-ml conical flask fitted with a ground-glass stopper. Add 300 ml of chloroform, measured at a definite temperature, replace the stopper, and shake mechanically for 4 to 5 hours. Set the flask aside overnight, and shake it for 30 minutes next morning.

Filter the solution through a fluted fast filter-paper, covering the top of the funnel to prevent loss of solvent, and collect 200 ml of filtrate (measured at the temperature at which the original 300 ml of chlorofom were measured). Transfer the solution to a 500-ml flask, and evaporate to dryness. Dissolve the residue in 100 ml of benzene - ether solution.

Transfer the solution to a pear-shaped 500-ml separating funnel with a further 20 ml of benzene - ether solution, and continue as described in the method for resin, beginning at "Cautiously add 50 ml of 2 per cent. potassium hydroxide solution. . . ."

CALCULATION—

The percentage of rotenone in the aliquot is calculated as described for the resin, *i.e.*, from the expression $10{\cdot}84\ W_1\ \alpha/W$. The percentage of rotenone in the root will therefore be obtained from the expression—

$$\text{Rotenone content, \%} = \frac{10{\cdot}84\, W_1\, \alpha}{W} \times \frac{3}{2} = \frac{16{\cdot}26\, W_1\, \alpha}{W}$$

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Notes

THE RAPID DETERMINATION OF EXCHANGEABLE CALCIUM AND MAGNESIUM IN HIGHLY LEACHED SOILS

SINCE Cheng and Bray[1] introduced ethylenediaminetetra-acetic acid (EDTA) as a reagent for soil analysis, several variations of their method have been suggested.[2] However, it is often stated or implied[3] that the removal of ammonium salts and interfering ions is necessary before the titrations with EDTA solution are carried out. The difficulty in directly titrating an extract arises largely because, for poor leached soils, the accuracy will be low, although interference by other elements may be absent, but for better soils, when the accuracy of a titration is sufficient, interfering elements are frequently encountered. Further, high concentrations of ammonium ions, such as occur in the most usual extracting solutions, tend to worsen the end-points.

The usual method is to titrate calcium and magnesium together at pH 10 with Eriochrome black T as indicator, and calcium alone at pH 12 with murexide as indicator. The more unsatisfactory end-point at low concentrations is given by murexide, and in the presence of high concentrations of ammonium ions it is impossible to obtain a reasonable accuracy by direct titration of extracts containing only a few parts per million of magnesium or calcium.

Several new indicators have been proposed as alternatives to murexide.[4,5,6,7] Two of these indicators (obtained from Hopkin and Williams Ltd.) have been qualitatively tested. Patton and Reeder's indicator[4] gave an excellent end-point, but appeared to be somewhat unstable in alkaline solution; the accuracy was poor unless the titration was carried out rapidly. Calcein[5] gave a rather unsatisfactory end-point under these conditions, although somewhat superior to that obtained when murexide was used. Another indicator, acid alizarin black SN, has recently been strongly recommended[8,9] and has now been tested on soil extracts in neutral *N* ammonium acetate. For dilute solutions, it was found to be much superior to murexide, and good accuracy could be obtained by direct titration even with poor soils.

EXPERIMENTAL

DETERMINATION OF CALCIUM—

The best pH for the use of acid alizarin black is between 12 and 12·5, although the titration can be carried out between pH 11·5 and 13. The colour change is from bright bluish pink to dull blue and the end-point is the point of maximum colour change. The colour continues to alter slightly on the further addition of EDTA, and as there is little warning of the approach of the end-point it may be overshot. A series of twenty titrations of a typical soil extract against 0·004 *N* EDTA solution was carried out over several days under different lighting conditions, the over-all standard error for one titration being $\pm 0{\cdot}06$ ml. Difficulty is experienced with acid alizarin black at high magnesium to calcium ratios, but such ratios are rarely found in highly leached soils.

The appropriate pH may be obtained by adding sodium hydroxide solution to the neutral *N* ammonium acetate extract, but this system is poorly buffered at about pH 12 and care is necessary when the alkali is added. The use of a hydroxide - borate buffer solution decreases the slope of the pH curve and is recommended. Diethylamine was tried, but did not give a sufficiently high pH under these conditions.

DETERMINATION OF CALCIUM *plus* MAGNESIUM—

Although alternative indicators, such as phthalein complexone,[10] have been suggested, Eriochrome black T gives equally good or better results. In presence of cyanide, a good end-point can be found by observing when the spots formed by added drops of reagent no longer contrast in colour with the solution. It is necessary to have a reasonable concentration of magnesium ions in the solution, and this is usually ensured by adding a solution of a magnesium salt that has been titrated to the Eriochrome black T end-point with EDTA solution. A series of twenty-five titrations carried out concurrently with the above-mentioned calcium titrations gave an over-all standard error of $\pm 0{\cdot}08$ ml of 0·004 *N* EDTA solution. A magnesium value obtained by difference therefore has a standard error equivalent to $\pm 0{\cdot}10$ ml. With the soil solution ratio used by us, 12·5 to 1, this gives a standard error of about $\pm 0{\cdot}01$ milli-equivalent of calcium and $\pm 0{\cdot}02$ milli-equivalent of magnesium per 100 g of soil.

These methods have been applied for some time in this laboratory, and good agreement has been obtained with determinations of calcium by flame photometry and of magnesium by using

Titan yellow. The recovery of calcium and magnesium added to typical soil extracts was complete, except at high magnesium to calcium ratios.

INTERFERING IONS—

The method has been designed on the assumptions that interference will not be serious for poor sandy soils and that the most important interfering ions will be NH_4^+, Fe^{3+}, Mn^{2+}, Al^{3+} and phosphate.

Ammonium—Interference by ammonium ions when Eriochrome black T is used as indicator appears as a dulling of the final blue colour and a slower colour change. However, it is not difficult to obtain an accurate end-point in solutions *N* in ammonium acetate at the end of the titration. At this concentration, the addition of potassium cyanide improves the sharpness of the end-point,[11] but this effect is small.

Ammonium ions have a similar effect when acid alizarin black is used as indicator, *i.e.*, the colour change is slower, but the end-point is adequate for concentrations of ammonium acetate up to *N* in the final solution. In 0·5 *N* ammonium acetate, the interference is extremely small.

Because of interference from ammonium ions, the end-points in both titrations may be slightly improved by diluting the solutions.

Iron—Iron interferes strongly when Eriochrome black T is used, partly by oxidising the indicator.[12] Up to 10 p.p.m. of iron can be effectively masked by cyanide, but at higher concentrations, up to about 30 p.p.m., sodium sulphide must be used[13]; the masking agent should be added well before the indicator. The interference appears as a dull reddish colour, which steadily increases at a rate dependent on the concentration of iron and prevents the appearance of the blue end-point colour.

Concentrations of iron up to 25 p.p.m. do not seriously interfere when acid alizarin black is used as indicator, but addition of cyanide may improve the end-point.

Manganese—This element rapidly oxidises Eriochrome black T, but the oxidation may be prevented by adding hydroxylamine hydrochloride. The manganese can then be quantitatively titrated, as magnesium *plus* calcium, the end-point being normal. The most effective way to remove manganese is by Cheng, Melsted and Bray's procedure,[14] the diethyldithiocarbamate complex being extracted with carbon tetrachloride.

This procedure is a disadvantage in a rapid method, but fortunately it need only be applied in the rare instances when manganese is present. At pH 7, manganese forms a red complex with Eriochrome black T, which, under these conditions, is just detectable at about 1 p.p.m. of manganese. If the indicator is added to the soil extract, with cyanide present, an immediate red or purple colour may be taken as an indication that manganese is present; high concentrations of iron and aluminium also give reddish colours, but more slowly. Owing to the variety of interfering effects that may occur in soil extracts, this test may not always be specific for manganese, but it should permit serious errors to be avoided. Titrations with and without the extraction procedure then give the amount of manganese present.

Concentrations of manganese below 25 p.p.m. do not seriously interfere when acid alizarin black is used as indicator.

Aluminium—Interference by aluminium when either Eriochrome black T or acid alizarin black is used can readily be overcome by triethanolamine,[12,15] which permits satisfactory titration in presence of at least 25 p.p.m. of aluminium. The masking agent is conveniently incorporated in the indicator solution.

Phosphate—Interference by phosphate is dependent on the concentration of calcium,[16] but, at the concentrations considered here (less than 0·004 *N*), at least 10 p.p.m. of phosphate can be present without interference when either indicator is used.

METHOD

REAGENTS—

EDTA solution, 0·004 N.

Eriochrome black T indicator solution—Dissolve 0·25 g of the dye (Colour Index No. 14,645) in 75 ml of triethanolamine, and dilute to 100 ml with water or ethanol to reduce the viscosity. If stored in a refrigerator, this solution is stable for a few months.

Sodium hydroxide - borate buffer solution—Dissolve 230 g of sodium hydroxide and 40 g of sodium tetraborate, both of analytical-reagent grade, in 2 litres of water. Set the solution aside, and filter if necessary.

Acid alizarin black indicator solution—Dissolve 0·5 g of the dye (Colour Index No. 21,725) in 75 ml of triethanolamine, and dilute to 100 ml with ethanol. Store in a refrigerator.

Magnesium - EDTA solution—With Eriochrome black T solution as indicator, titrate a portion of 0·004 *N* magnesium solution to the end-point with 0·004 *N* EDTA solution.

PROCEDURE FOR DETERMINING CALCIUM—

To 25 ml of soil extract in neutral *N* ammonium acetate add 10 ml of sodium hydroxide - borate buffer solution. Check the pH with a new batch of buffer solution or a different type of soil, and adjust to between 12 and 12·5. Add 10 drops of acid alizarin black indicator solution, as required, and titrate the bright bluish pink solution with 0·004 *N* EDTA solution until the red colour suddenly fades. Carry out a blank titration, as the buffer solution usually contains appreciable amounts of calcium.

PROCEDURE FOR DETERMINING CALCIUM *plus* MAGNESIUM (*plus* MANGANESE IF PRESENT)—

To 25 ml of soil extract add about 0·05 g of analytical-reagent grade potassium cyanide, set aside, and then add 6 to 8 drops of Eriochrome black T indicator solution. If the solution is a clear blue, add 2 ml of magnesium - EDTA solution and 8 ml of ammonia solution, sp.gr. 0·880, and titrate to a clear light blue or blue-green colour.

If, when the indicator solution is added, a purple or red colour appears at once, manganese is present. Add about 0·01 g of analytical-reagent grade hydroxylamine hydrochloride, heat to about 70° C, add the other reagents, as described in the preceding paragraph, and titrate for calcium *plus* magnesium *plus* manganese. Place another 25-ml portion of soil extract in a separating funnel, and add 1 drop of concentrated hydrochloric acid and about 20 mg of sodium diethyldithiocarbamate. Extract with carbon tetrachloride until the extract is colourless. Transfer the aqueous layer quantitatively to a flask, and titrate as before for calcium *plus* magnesium.

If the solution changes colour gradually during the titration and no proper end-point is obtained severe contamination by iron is evident. In this event, add a little sodium sulphide with the potassium cyanide, and titrate as rapidly as possible.

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DETERMINATION OF TRACES OF CYANIDE IN FISH TISSUE

Cyanide can be recovered from poisoned fish tissue by steam-distilling the acidified material into sodium hydroxide solution, although this method has the disadvantage that the distillate may be turbid, particularly if the tissue is in a putrid condition. Kruse and Mellon[1] state that turbidity can be removed by extraction with a solvent such as *iso*octane, but this method is not always effective for distillates from putrid fish tissue. Interference from turbidity can be obviated by using Conway's microdiffusion technique.[2,3] A high degree of sensitivity can be attained by determining the recovered cyanide with a pyridine - pyrazolone reagent solution.[4] Filaments excised from the gills of fish submitted for examination were found to be the most convenient material for analysis.

Method

Reagents—

Sodium hydroxide, 0·1 N.

Acetic acid, 0·5 *per cent. v/v.*

Sulphuric acid, 10 *per cent. v/v.*

Chloramine T solution, 1 *per cent. w/v, aqueous*—Freshly prepared.

Pyridine - pyrazolone reagent solution—Dissolve 0·025 g of bispyrazolone in 25 ml of pyridine, and mix the solution with 125 ml of a saturated aqueous solution of 1-phenyl-3-methyl-5-pyrazolone. This reagent was always used within 24 hours of preparation.

Procedure—

Cut up the gill filaments, and homogenise a weighed portion of chopped material with 5 times its weight of water in a laboratory homogeniser. Transfer 3 g of homogenate to a Conway cell (size No. 1) containing 2 ml of 0·1 *N* sodium hydroxide in the inner chamber. Acidify the homogenate with 1 ml of 10 per cent. v/v sulphuric acid that has been warmed to 30° C, rock the unit to mix, and set aside for 3 hours to ensure complete diffusion. Transfer a 1-ml aliquot from the inner chamber to a 50-ml stoppered tube, add 1·3 ml of 0·5 per cent. v/v acetic acid (which decreases the pH to approximately 5·0), and adjust the volume to 15 ml with water. Add 0·2 ml of 1 per cent. w/v chloramine T solution, invert to mix, set aside for 1½ minutes, add 5 ml of pyridine - pyrazolone reagent solution, and again mix by inversion. After 30 minutes (the time required for optimum colour development) dilute the contents of the tube to 50, 100 or 200 ml, depending on the colour intensity, and measure the optical density at 630 mμ in a 10-cm cell with an absorptiometer. Determine the amount of cyanide present from a previously prepared calibration graph. Note that it is advisable to carry out a reagent-blank test with each series of determinations, as this value increases with the storage time of the pyridine - pyrazolone reagent solution.

Table I

Recovery of added cyanide from healthy tissue

Cyanide added, μg	Total cyanide recovered, μg	Added cyanide recovered, μg	Recovery, %
0·000	0·014	—	—
0·000	0·014	—	—
0·500	0·504	0·490	98·0
0·500	0·508	0·494	98·8
1·000	0·998	0·984	98·4
1·000	0·974	0·960	96·0
1·500	1·504	1·490	99·4
1·500	1·490	1·476	98·4
2·000	1·934	1·920	96·0
2·000	1·960	1·946	97·3
2·000	1·960	1·946	97·3
2·000	1·968	1·954	97·7

Results

Table I shows the recoveries obtained when measured amounts of a standard cyanide solution were added to a sample of homogenate prepared from the gills of healthy fish.

The mean recovery was 97·7 per cent., and the standard error 1·11 per cent. At the 5 per cent. level of significance the mean recovery does not differ significantly from that obtained for cyanide solutions in the absence of tissue homogenate.

Discussion of the method

A modification of the standard Conway cell has been used in the analysis of unpoisoned fish tissue, in which low cyanide contents were expected. This cell was made by using three Petri-dish lids, two having diameters of 10 cm and one a diameter of 5·5 cm. The small dish was held inside one of the larger ones by a smear of petroleum jelly; the third dish was used as a lid for the unit and was sealed to the lower portion by a broad rubber band around the periphery. The cell held 30 g of homogenate and 4 ml of sulphuric acid in its outer chamber, and 8 ml of sodium hydroxide solution were placed in the inner chamber. Diffusion was allowed to continue overnight. The pH of a 4-ml aliquot from the inner chamber was adjusted by adding 5·2 ml of acetic acid, and the solution was diluted to 15 ml with water. Colour was developed and measured in the way just described.

Recoveries from this modified cell did not differ significantly from those obtained with the standard unit, provided that, in the modified cell, the level of the contents of the outer chamber was within 5 mm of the rim of the inner chamber. Determinations with standard cyanide solutions were carried out in both types of cell to demonstrate the effect of warming the sulphuric acid to 30° C. When sulphuric acid at room temperature was used, recovery was $96{\cdot}9 \pm 3{\cdot}0$ per cent., whereas if the acid were warmed to 30° C, recovery was $99{\cdot}6 \pm 2{\cdot}6$ per cent. The standard deviations are those of a single determination. These recoveries do not differ at the 5 per cent. level of significance.

To indicate the occurrence of cyanide poisoning in a river, the tissues of more than one fish must be analysed, and it is recommended that a sample of at least five fish (preferably found dead in the water and not on the bank) should be used, as may be seen from the following results. The modified cell was used in the analysis of samples from thirty-eight salmon that had not been poisoned; the concentration of cyanide in the gill filaments ranged from 0·002 to 0·107 p.p.m. The values showed a lognormal distribution, the mean being 0·015 p.p.m., and only one exceeded the upper 95 per cent. confidence limit of 0·067 p.p.m. Of thirty fish believed to have been poisoned, the gills of only three contained less than 0·067 p.p.m. of cyanide, the values ranging from 0·045 to 0·376 p.p.m. The correct method of comparing the two sets of results discussed above would be to compare mean logarithmic values. Comparison of individual results from suspect fish with the upper 95 per cent. confidence limit for unpoisoned fish has been proposed, on the grounds that an analyst may not receive a sufficient number of suspect fish to permit accurate statistical assessment. A sample of fish may contain a number of specimens that, for various reasons, have low concentrations of cyanide, and the mean of a small sample of large variance may not differ significantly from that of the unpoisoned range. High values in a part of the sample would normally be sufficient evidence of poisoning.

Salmon exposed to water at 4° C containing 50 p.p.m. of cyanide until death occurred (20 to 30 minutes) had about 2·5 p.p.m. of cyanide in the gills when analysed 4 hours after death, but the concentration had decreased to about 0·5 p.p.m. in the gills of fish that had been stored for 3 days at 20° C. The interval between death and analysis should therefore be kept to a minimum, and losses will also be reduced if the tissues are refrigerated during this period.

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A NEUTRAL REAGENT FOR THE ROUTINE DETERMINATION OF FAT IN SEPARATED MILK AND LOW-FAT PRODUCTS

The neutral reagent solution previously described[1] was not suitable for determining fat in separated milk or in products having a low fat content, but observations during that work suggested that the reagent could be suitably modified by substituting the polyoxyethylene

derivative of sorbitol monolaurate (Tween 21) for the polyoxyethylene derivative of sorbitol trioleate (Tween 85).

Method

Apparatus—

Use the apparatus specified for determining fat in milk and milk products by the Gerber method,[3] but use the skim-milk butyrometer instead of the standard milk butyrometer.

Preparation of reagent solution—

The reagent solution has the following composition—

Trisodium citrate, 5 per cent. w/v;
Sodium salicylate, 5 per cent. w/v;
Disodium ethylenediaminetetra-acetate, 1 per cent. w/v;
Tween 21, 0·5 per cent. w/v;
Industrial methylated spirit (66 O.P.), 25 per cent. v/v.

Weigh the Tween (obtainable from Honeywill - Atlas, Devonshire House, Mayfair Place, Piccadilly, London, W.1) into a small beaker, dissolve it in part of the methylated spirit, transfer to a calibrated vessel, and use the remainder of the spirit to rinse the beaker. Dissolve the disodium ethylenediaminetetra-acetate in hot water, and neutralise the solution to phenolphthalein with *N* sodium hydroxide. Dissolve the citrate and salicylate in this mixture, and then add it to the mixture in the calibrated vessel. Cool to 20° C, dilute to the mark with water, and mix.

The reagent solution is an opalescent liquid having a pH of 7·8. To distinguish it from the reagent for full-cream products, it can be coloured green by the addition of a little naphthol green BNS (obtainable from Imperial Chemical Industries Ltd.).

Procedure for separated, low-fat or chocolate-flavoured milk—

Measure 10 ml of the reagent solution into a skim-milk butyrometer, and add 10·94 ml of well mixed sample by pipette. Close the butyrometer with a stopper, invert twice, and place in a water bath at 65° C for 5 minutes. Shake the butyrometer until the contents are thoroughly mixed, and invert it two or three times while shaking. Spin the butyrometer in the centrifuge at 1100 r.p.m. for 5 minutes immediately after mixing. Place the butyrometer, stopper downwards, in the bath at 65° C for at least 3 minutes, and read the percentage of fat. Repeat the spinning, warming and reading until two consecutive readings are the same.

Correct the final observed reading as follows—

Reading less than 0·10 per cent., add 0·05 per cent.
Reading 0·10 to 0·25 per cent., add 0·02 per cent.
Reading more than 0·25 per cent., no correction.

Procedure for buttermilk and fat-free yoghourt—

Weigh 11·3 $\pm$ 0·05 g of sample into a small dish, and transfer to a skim-milk butyrometer by pouring down a glass rod. Use 10 ml of reagent solution in two portions to rinse the remainder of the sample into the butyrometer. Complete the test as for separated milk, and apply any appropriate correction to the butyrometer reading.

Procedure for cottage cheese—

Weigh 3 $\pm$ 0·01 g of sample into a small dish. Add 4 ml of warm water, and stir to disperse the cheese. Transfer to a skim-milk butyrometer containing 10 ml of reagent solution, and rinse the dish with two 2·5-ml portions of warm water. Add the rinsings to the contents of the butyrometer, and complete the test as for separated milk (shake the mixture until all particles of curd have dissolved).

Fat content, per cent. = Butyrometer reading $\times$ 3·9.

Results

Fat contents found by the proposed method were compared with those found by established methods. The Gerber method[3] was used as a reference for separated milk, low-fat milk, chocolate-flavoured milk, buttermilk and yoghourt, and the gravimetric method[4] was used for cheese. The results are summarised in Table I.

Conclusions

A neutral reagent solution has been devised; it can be used with standard Gerber apparatus to determine the fat content of separated milk, low-fat milk, chocolate-flavoured milk, buttermilk, fat-free yoghourt and cottage cheese with an accuracy equal to that of the Gerber method. It possesses certain advantages over acid in that it does not attack the skin or clothing and is non-corrosive to metals and glassware; it causes no charring or discoloration when used with sweetened products. The solution is neutral and does not hydrolyse the fat on prolonged contact.

Table I

Frequency distribution of differences between results by proposed and reference methods

Product	Range of fat content, %	No. of samples tested	Difference between fat contents by proposed and reference methods, %	No. of results with this difference	Mean difference in fat content, %
Separated milk	0·07 to 0·22	25	+0·05 to +0·06	0	−0·006
			+0·03 to +0·04	0	
			0 to +0·02	9	
			−0·02 to 0	16	
			−0·03 to −0·04	0	
			−0·05 to −0·06	0	
Low-fat milk	0·3 to 1·0	50	+0·05 to +0·06	0	−0·006
			+0·03 to +0·04	0	
			0 to +0·02	19	
			−0·02 to 0	26	
			−0·03 to −0·04	5	
			−0·05 to −0·06	0	
Chocolate-flavoured milk	1·0 to 1·4	24	+0·05 to +0·06	0	−0·036
			+0·03 to +0·04	0	
			0 to +0·02	0	
			−0·02 to 0	5	
			−0·03 to −0·04	19	
			−0·05 to −0·06	0	
Buttermilk	0·9 to 1·6	24	+0·05 to +0·06	1	−0·003
			+0·03 to +0·04	2	
			0 to +0·02	8	
			−0·02 to 0	8	
			−0·03 to −0·04	5	
			−0·05 to −0·06	0	
Free-fat yoghourt ..	0·13 to 0·80	12	+0·05 to +0·06	0	−0·007
			+0·03 to +0·04	0	
			0 to +0·02	4	
			−0·02 to 0	6	
			−0·03 to −0·04	2	
			−0·05 to −0·06	0	
Cottage cheese	4·2 to 5·2	12	+0·11 to +0·15	1	+0·006
			+0·06 to +0·10	2	
			0 to +0·05	3	
			−0·05 to 0	4	
			−0·06 to −0·10	2	
			−0·11 to −0·15	0	

I thank Miss Irene Brookes for technical assistance, and Honeywill - Atlas Ltd. for providing samples of Tween.

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THE ASSAY OF L-PHENYLALANINE IN CASEIN HYDROLYSATES

VARIOUS methods, chemical, enzymatic and microbiological, exist for determining phenylalanine. With the production of special phenylalanine-low casein hydrolysates for the treatment of phenylketonuria, the need for a rapid and sensitive method of assay has led to an examination of the available procedures. We have compared results by—

(*i*) the decarboxylase method,[1] in which L-phenylalanine is assayed as phenylethylamine by a modification of Brodie and Udenfriend's methyl orange procedure[2];
(*ii*) nitration by the Kapeller - Adler - Kuhn method and then hydroxamic acid formation[3];
(*iii*) a microbiological plate assay with a mutant of *Escherichia coli* and the special media described by Dickinson[4] and Jones and Burns[5];
(*iv*) Horn, Jones and Blum's microbiological tube-dilution assay,[6] in which *Leuconostoc mesenteroides* P60 is used.

EXPERIMENTAL

Hydrolysates were prepared by heating casein under reflux for 24 hours with 6 *N* hydrochloric acid and were then treated with charcoal to give different phenylalanine contents. In the microbiological determinations, the plate method was first used, and the assay ranges for the tube method were chosen according to the results of the plate test. For the other methods, the approximate phenylalanine contents were determined by paper chromatography in a *n*-butyl alcohol - ethanol - water mixture.[7] No indication was given to the analysts of the probable levels of phenylalanine in the samples, which were submitted in random order of concentrations. Casein hydrolysates containing from 0·02 to 10 per cent. of L-phenylalanine were examined. The sensitive decarboxylase method was able to detect 2 μg of the amino acid, but, when possible, the amount of sample taken was adjusted to contain between 10 and 50 μg of L-phenylalanine. When the modified Kapeller - Adler - Kuhn method was used, between 0·5 and 2 mg of phenylalanine were required, and this insensitivity gave rise to difficulties in the assay of casein hydrolysates containing less than 1 per cent. of phenylalanine. The levels used in the plate assay were similar to those in the decarboxylase method. The tube-dilution method was able to detect 1 to 2 μg of L-phenylalanine.

TABLE I

COMPARISON OF RESULTS FOR L-PHENYLALANINE

Sample No.	L-Phenylalanine found by— decarboxylase method, %	Kapeller - Adler - Kuhn method, %	plate assay, %	tube-dilution assay, %	Calculated L-phenylalanine content, %
Casein hydrolysates plus *known amounts of* DL-*phenylalanine*—					
1	9·4	9·4	9·2	9·2	9·3
2	3·3	3·4	3·2	3·4	3·3
3	2·3	2·3	2·3	2·3	2·2
4	0·98	1·0	0·96	1·0	0·98
5	0·45	0·52	0·44	0·42	0·42
6	0·12	0·21	0·14	0·13	0·13
7	0·03	—	0·02	0·02	0·02
Processed casein hydrolysates—					
8	8·4	—	8·2	8·4	—
9	4·7	—	4·6	4·8	—
10	2·4	—	2·4	2·6	—
11	1·3	—	1·3	1·3	—
12	0·5	—	0·5	0·5	—
13	0·1	—	0·1	0·1	—
14	8·5	8·5	8·5	8·4	—
15	3·9	4·0	4·1	3·9	—
16	2·1	2·0	2·0	2·0	—
17	1·1	1·0	1·3	1·0	—
18	0·25	0·34	0·25	0·23	—
19	0·03	—	0·02	<0·01	—

DISCUSSION OF RESULTS

The results in Table I are for two types of sample. Samples Nos. 1 to 7 were solutions of casein hydrolysates containing 0·02 per cent. of L-phenylalanine to which had been added known

amounts of DL-phenylalanine; samples Nos. 8 to 19 were casein hydrolysates that had been processed so as to have different phenylalanine contents.

In the biological methods it was found that D-phenylalanine did not interfere until the D to L ratio exceeded 4 to 1, whereas both isomers were determined by the nitration method.

The decarboxylase method involves preparation of an acetone-dried powder of *Streptococcus faecalis* at frequent intervals, which is time-consuming. This method, however, is simple, rapid and sensitive. In comparison, the Kapeller - Adler - Kuhn method is insensitive, although it is reliable for normal casein hydrolysates. The tube-dilution assay is expensive and laborious. The plate method has the advantages of rapidity and simplicity, and our results show that when the modified medium[4,5] is used it provides a reliable assay for casein hydrolysates (including those of the special phenylalanine-low caseins now in use for phenylketonuria).

We acknowledge the gift of a culture of *S. faecalis* from Dr. L. I. Woolf, Great Ormond Street Hospital for Sick Children, London, and the competent technical assistance of D. Smith. We thank the Directors of Beecham Research Laboratories Ltd. and Beecham Maclean Ltd. for permission to publish this Note.

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THE DETERMINATION OF CYANIDE IN EFFLUENTS

The method recommended by the Joint Committee of the Association of British Chemical Manufacturers and the Society for Analytical Chemistry for determining small amounts of cyanide in trade effluents[1] is based on the method developed by Aldridge.[2] In this method, cyanide and thiocyanate are determined together and thiocyanate is then determined in a separate portion of the acidified and aerated sample; the cyanide content is obtained by difference. If an effluent contained a relatively large amount of thiocyanate, *e.g.*, 20 p.p.m., and only a small but significant amount of cyanide, *e.g.*, 1 p.p.m., any error in either determination would affect the cyanide result. Such an error, although of little significance on the thiocyanate content, especially as this is not a toxic substance, could greatly affect the result of the cyanide determination. Because of this, and also because Aldridge's method cannot readily be applied to coloured effluents, we attempted to determine cyanide directly in presence of thiocyanate, ferrocyanide and ferricyanide.

Conditions have been established whereby cyanide can be distilled from a solution also containing thiocyanate, ferrocyanide and ferricyanide, the last two compounds being fixed with zinc acetate. The results show that up to 3 p.p.m. of CN, present as cyanide, can be accurately determined in presence of up to 25 p.p.m. of CN, present as thiocyanate, and up to 5 p.p.m. of CN, present as ferrocyanide or ferricyanide, in aqueous solutions containing all these substances and also in presence of sodium and calcium chlorides. The proposed method is accurate to within $\pm 0{\cdot}05$ p.p.m. of CN at the maximum concentrations of these substances and to within $\pm 0{\cdot}01$ p.p.m. of CN at lower concentrations. A wide range of contents of the various cyanides has been covered by the experimental work.

Colour is developed in a much larger volume of solution than that used by Aldridge; this permits absorptiometric or spectrophotometric measurements to be made in 4-cm cells and thus increases the sensitivity of the method. The colorimetric finish—a modification to Aldridge's

method—was described in a private communication from Mr. H. N. Wilson (Division Chief Analyst, Imperial Chemical Industries Ltd., Billingham), who used it to determine cyanide in aqueous solutions containing cyanide, thiocyanate, ferrocyanide and ferricyanide; the solutions were eluates from a chromatographic separation on an alumina column.

Method

Reagents—

Hydrochloric acid, N and 0·1 N.

Sodium hydroxide, 0·1 N.

Methyl red indicator solution, 0·04 per cent. w/v.

Bromine water—A saturated solution.

Zinc acetate solution, 10 per cent. w/v—Dissolve 50 g of zinc acetate, $Zn(C_2H_3O_2)_2.2H_2O$, in 400 ml of distilled water, filter, and dilute to 500 ml.

Arsenious acid solution—Heat 2 g of arsenious oxide and 100 ml of distilled water under reflux until solution is complete, cool, and filter.

Pyridine - benzidine hydrochloride solution—Carefully add 50 ml of hydrochloric acid, sp.gr. 1·18, with stirring, to 350 ml of redistilled pyridine, and allow to cool. Heat 3·7 g of benzidine with 50 ml of *N* hydrochloric acid in a fume cupboard until solution is complete, cool, and dilute to 100 ml with distilled water. Just before use, prepare the required amount of reagent by mixing 8 volumes of the pyridine solution with 2 volumes of the freshly filtered benzidine solution.

Standard cyanide solution—Determine the purity of a sample of potassium cyanide by titration against 0·1 *N* silver nitrate, and then prepare a stock solution containing 100 μg of CN per ml. Dilute a portion of this solution until it contains 1·0 μg of CN per ml.

Procedure—

Measure 20 ml of the filtered sample into a 250-ml distillation flask, and dilute to 90 ml with distilled water. Add 2 drops of methyl red indicator solution, neutralise with 0·1 *N* hydrochloric acid, and then add 10 ml of 0·1 *N* hydrochloric acid from a burette; mix the solution gently during the addition. Add 10 ml of zinc acetate solution, place a small boiling-rod in the flask, and attach an all-glass water-cooled condenser. (The condenser should be at a slight angle from the horizontal and have a delivery end about 9 inches long; the end attached to the flask should be about 5 inches long and ½ inch in diameter.) Set aside for 5 minutes. In each of three 50-ml stoppered graduated cylinders place 5 ml of 0·1 *N* sodium hydroxide. Distil the cyanide by gently boiling the solution in the distillation flask over a small bunsen flame, and collect the hydrogen cyanide in the contents of one of the 50-ml cylinders. (Ensure that the delivery end of the condenser dips beneath the surface of the sodium hydroxide solution.) Collect about 25 ml of distillate, replace the cylinder with a second cylinder, and continue distillation until a further 25 ml of distillate have been collected. Replace this cylinder with the third cylinder, and collect a further 25 ml of distillate.

Determine the cyanide in the contents of each cylinder as described below.

Adjust the temperature of the solution to between 18° and 20° C, add 1 drop of methyl red indicator solution, neutralise with *N* hydrochloric acid, and add 1 drop of acid in excess. Add 1 ml of bromine water, mix, and set aside for 5 minutes. Remove the excess of bromine by adding 1 ml of arsenious acid solution, mix by inverting the stoppered cylinder, and again set aside for 5 minutes.

Add 10 ml of pyridine - benzidine hydrochloride solution, dilute to the 50-ml mark with distilled water, and mix thoroughly. Set aside in the dark for 20 minutes to attain full colour development.

Measure the optical density of each solution against distilled water with a Spekker absorptiometer. Use either a 1- or a 4-cm cell, according to the depth of colour of the first portion of distillate, and Ilford No. 604 (green) filters in conjunction with H503 heat filters. Correct the indicator-drum readings for the reagent-blank value, and, from the net reading for each solution, obtain the total amount of cyanide, as micrograms of CN, from a previously prepared calibration graph. Calculate the cyanide content of the sample from the expression—

$$\text{Cyanide present, as CN, p.p.m.} = \frac{\text{Amount of cyanide in distillate, } \mu g}{\text{Volume of sample, ml}}$$

PREPARATION OF CALIBRATION GRAPHS—

For amounts of cyanide up to 4 μg—In a series of 50-ml stoppered graduated cylinders place amounts of standard cyanide solution representing 0·0, 1·0, 2·0, 3·0 and 4·0 μg of cyanide. Dilute each solution to 30 ml with distilled water, add 1 drop of methyl red indicator solution, neutralise with 0·1 *N* hydrochloric acid, and add 5 drops of acid in excess. Continue as described under "Procedure," beginning at "Add 1 ml of bromine water. . . ."

Measure the optical density of each solution in a 4-cm cell, deduct the blank value from each reading, and plot the net indicator-drum readings against the amounts of cyanide present.

For amounts of cyanide from 4 to 15 μg—In a series of 50-ml stoppered graduated cylinders place amounts of standard cyanide solution representing 0·0, 4·0, 5·0, 6·0, 7·5, 10·0, 12·5 and 15·0 μg of cyanide, and continue as described above. Measure the optical densities in 1-cm cells.

TABLE I

AMOUNTS OF CYANIDE FOUND IN VARIOUS AQUEOUS SOLUTIONS

KCNS added, as CN, μg	$K_4Fe(CN)_6$ added, as CN, μg	$K_3Fe(CN)_6$ added, as CN, μg	Amount of cyanide found— in first 25 ml of distillate, μg	in second 25 ml of distillate, μg	in third 25 ml of distillate, μg	Total cyanide found, μg
In absence of sodium and calcium chlorides—						
20	5	5	0·02	Nil	Nil	0·02
50	10	10	0·02	Nil	Nil	0·02
100	50	50	0·10	0·05	0·05	0·20
In presence of 1 g each of sodium and calcium chlorides—						
20	5	5	0·02	Nil	Nil	0·02
50	10	10	0·02	Nil	Nil	0·02
100	50	50	0·05	0·01	Nil	0·06

TABLE II

RECOVERY OF ADDED CYANIDE FROM AQUEOUS SOLUTIONS

KCNS added, as CN, μg	$K_4Fe(CN)_6$ added, as CN, μg	$K_3Fe(CN)_6$ added, as CN, μg	KCN added, as CN, μg	Amount of cyanide found*— in first 25 ml of distillate, μg	in second 25 ml of distillate, μg	in third 25 ml of distillate, μg	Total cyanide found, μg
In absence of sodium and calcium chlorides—							
50	10	10	2·0	1·56	0·35	0·07	1·98
			5·0	3·80	1·00	0·30	5·10
			10·0	7·70	1·83	0·42	9·95
100	50	50	2·0	1·60	0·47	0·20	2·27
			5·0	4·00	1·05	0·23	5·28
			10·0	7·50	1·88	0·54	9·92
In presence of 1 g each of sodium and calcium chlorides—							
20	5	5	1·0	0·81	0·09	0·04	0·94
50	5	5	3·0	2·51	0·37	0·04	2·92
			5·0	4·15	0·60	0·10	4·85
			10·0	8·30	1·17	0·19	9·66
			15·0	12·80	1·70	0·30	14·80
100	50	50	1·0	0·93	0·09	0·04	1·06
			3·0	2·65	0·34	0·08	3·07
			5·0	4·10	0·60	0·08	4·78
			10·0	8·50	1·32	0·25	10·07
			15·0	12·80	1·75	0·35	14·90

* Corrected for blank value.

RESULTS

Table I shows the results obtained when aqueous solutions containing known amounts of potassium thiocyanate, ferrocyanide and ferricyanide, in presence and absence of sodium and calcium chlorides, were analysed by the proposed procedure; the recoveries of added cyanide from similar solutions are shown in Table II.

A sample of an effluent containing calcium and sodium chlorides was filtered and the filtrate was stored in a dark-coloured bottle. Known amounts of potassium cyanide were added to 20-ml portions of the filtrate, which were then analysed by the proposed procedure; the results are shown in Table III.

TABLE III

RECOVERY OF CYANIDE ADDED TO AN EFFLUENT

KCN added, as CN, μg	Amount of cyanide found, μg	Amount of added cyanide recovered, μg	Amount of cyanide found, p.p.m.
Nil	2·03	—	0·10
2·0	4·00	1·97	0·20
5·0	7·05	5·02	0·35
10·0	12·20	10·17	0·61
15·0	16·80	14·77	0·84

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2. Aldridge, W. N., *Analyst*, 1944, **69**, 262; 1945, **70**, 474.

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Book Reviews

A PROPOSED INTERNATIONAL CHEMICAL NOTATION. Prepared by the Commission on Codification, Ciphering and Punched Card Techniques of the International Union of Pure and Applied Chemistry. Tentative Version; subject to revision. Pp. viii + 165. London, New York and Toronto: Longmans, Green & Co. Ltd. 1958. Price 35s.

G. M. Dyson proposed a system for the notation and enumeration of organic compounds in the autumn of 1946, and this was published next year and reviewed in *The Analyst*, 1947, **72**, 176. The system was based on the principle of breaking down the structure to individual rings, chains, functional groups, etc., and citing these in a fixed order by conventional symbols. At that time it proved possible to detail the system on two pages of *The Analyst* (1947, **72**, 155). Another system was published by F. L. Taylor in the U.S.A. in 1948, and the two systems were later reconciled. Others have since appeared, some of similar approach to the first two, others quite different. All of these have been reviewed by the Commission of the International Union, which started work early in 1947 and early laid down a comprehensive set of desiderata for internationally acceptable cipher notation. In Paris in 1957 the Commission finally accepted a draft based on the Dysonian Notation, and this volume is the official exposition of the accepted system. It is no longer possible to abstract it, as before, in two pages of *The Analyst*; indeed, it is doubtful if it could now be compressed into much less than the 144 pages it occupies in this book. But the system has emerged most successfully from the developments of the last decade, absorbing criticisms and suggestions on the way, and it now stands as the future basis of indexes and mechanical and electronic techniques for the delineation of exact organic chemical structure; it is a truly international means for stating such structures independently of national language.

K. A. WILLIAMS

A LABORATORY MANUAL OF CHEMICAL PATHOLOGY. By Professor F. T. G. PRUNTY, Dr. R. R. MCSWINEY and Dr. JOYCE B. HAWKINS. Pp. x + 260. London, New York, Paris and Los Angeles: Pergamon Press Ltd. 1959. Price 35s.

This is a practical manual containing detailed accounts of the methods used in the Chemical Pathology Department of St. Thomas's Hospital Medical School. No attempt has been made to review all the techniques available for the estimation of the various substances measured, and

the interpretation of the results is not discussed. This policy of judicious exclusion has produced a refreshingly concise volume of useful practical information. Methods for the measurement of seventy-three body constituents are given, and the account of each determination includes details of reagent preparation, analytical technique and calculation. Although the book is sufficiently comprehensive to include methods for blood ammonia, magnesium and hexosamines, it is surprising to find no mention of serum transaminase determinations and of other recently developed methods for blood enzymes. The inclusion of the glucose oxidase technique for blood glucose is very commendable, but I would prefer more convenient methods for salicylates, phosphatases and faecal fat. A most useful section is that dealing with the collection, homogenising and analysis of food, faeces and urine in various metabolic balances. The choice of methods is lucidly discussed in the introductory chapter, and the use of standard solutions and of the quality-control system in reducing laboratory errors is rightly emphasised. The authors must be congratulated for compiling such a sensible collection of methods; the book should be found in every department of clinical chemistry.

M. J. H. SMITH

RIVER POLLUTION: 1. CHEMICAL ANALYSIS. By LOUIS KLEIN, M.Sc., Ph.D., F.R.I.C., M.Inst.S.P. Pp. x + 206. London: Butterworths Scientific Publications. 1959. Price 30s.

In this book Dr. Klein has collected together and extended chapters 9 and 10 of his larger work, "Aspects of River Pollution," which was reviewed in *The Analyst*, 1957, **82**, 715. The result is not a textbook on water analysis, but a review that includes well documented references to almost every important method in this specialised field. It differs from its parent chapters, as children will, largely in the modernity of its information.

Under the pressure inescapable in most laboratories where large numbers of samples of any one general type are analysed, there is inevitably a tendency to rely too much upon long-established routine methods and to postpone indefinitely a time-consuming re-assessment of them in the light of the overwhelming volume of new publications. Dr. Klein has produced an excellent reference book that will minimise the labour of this rewarding exercise.

J. G. SHERRATT

THE SEQUESTRATION OF METALS: THEORETICAL CONSIDERATIONS AND PRACTICAL APPLICATIONS. By ROBERT L. SMITH, B.Sc., Ph.D., F.R.I.C. Pp. viii + 256. London: Chapman and Hall Ltd. 1959. Price 42s.

The tactful curate is said to have eulogised his bad egg in the famous phrase "Parts of it are excellent." The same may be said of the present book, for the later chapters describing the applications of sequestering agents in industry, in analytical chemistry and in applied biology are packed with information and a great many useful references to original literature.

For the rest, one can be but amazed that so many errors have found their way into print. Typographical errors abound and there are over 60 in the first 100 pages. Unfortunately, these are most frequent where they are least excusable, *i.e.*, in proper names, in formulae and in equations. Sometimes plus and minus signs (for addition or subtraction) are omitted (pp. 35 and 65), elsewhere they are used where superscripts or subscripts are intended (see p. 67, where there are seven errors in 2 lines). This may be explained, but not excused, by hasty proof-reading. But there is no such justification for the misuse or abuse of technical terms such as appears in the following extracts—"ethylene diamine and its *polymeric* forms"; "unfavourable positions . . . make piperidine a poor *chelating* agent"; "if the *substitution* is methylamine"; "The polyamines with a *rigidity of structure* and possibly assisted by *resonance* factors such as . . . and also for that matter 1,2-diaminocyclohexane. . . ." (my italics).

The author appears to have seriously underestimated the background knowledge of the "chemist of graduate, A.R.I.C. or similar status" to whom this book is addressed. In "writing down" to their supposed level, the author has often produced unsatisfactory accounts of physical processes involving solvation, chelation, entropy and mesomerism. The over-simplification has sometimes led to arrant absurdity, as when we are told that in the ionisation of a dicarboxylic acid (p. 12) ". . . neither group entirely parts with a proton. Instead they both lose a portion —of a proton."

A book on the sequestration of metals was badly needed. The present volume does not meet the need adequately, and its circulation can scarcely enhance the reputation of the author or of the publishers.

H. IRVING

Publications Received

TREATISE ON ANALYTICAL CHEMISTRY. Edited by I. M. KOLTHOFF and PHILIP J. ELVING, with the assistance of ERNEST B. SANDELL. Part I. THEORY AND PRACTICE, Vol. 1. Pp. xxvi + 809. New York and London: Interscience Publishers Inc. 1959. Price 133s.; $17.50.

CIGARETTE SMOKE CONDENSATE: PREPARATION AND ROUTINE LABORATORY ESTIMATION. Research Paper No. 4. By H. R. BENTLEY and J. G. BURGAN. Pp. iv + 9. London: Tobacco Manufacturers' Standing Committee. 1959. Gratis.

The term "tar," which has been widely used in connection with cigarette smoke, is replaced by "smoke condensate," a term having no absolute meaning and therefore entirely a matter of definition. A standard method is proposed for the selection of cigarettes for testing, for their smoking in an automatic machine, and for the estimation of the smoke condensate produced. The method is based on a survey of the smoking habits of individuals and has been chosen to provide a maximum degree of reproducibility and ease of treatment in analysis.

AMMONIA MANUFACTURE AND USES. By A. J. HARDING, M.A., Ph.D. Pp. xiv + 41. *Published under the auspices of Imperial Chemical Industries Ltd.* London, New York and Toronto: Oxford University Press. 1959. Price 6s. 6d.

ELECTROLYTIC MANUFACTURE OF CHEMICALS FROM SALT. By D. W. F. HARDIE, B.Sc., Ph.D. Pp. xii + 74. *Published under the auspices of Imperial Chemical Industries Ltd.* London, New York and Toronto: Oxford University Press. 1959. Price 7s. 6d.

ANNUAL REPORTS ON THE PROGRESS OF CHEMISTRY FOR 1958. Vol. LV. Pp. 527. London: The Chemical Society. 1959. Price 40s.

ION EXCHANGE RESINS. Fourth Edition. Pp. iv + 68. Poole, Dorset: The British Drug Houses Ltd.

PRINCIPLES AND PRACTICE OF GAS CHROMATOGRAPHY. Edited by ROBERT L. PECSOK. Pp. xii + 226. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1959. Price $6.75; 54s.

GAS CHROMATOGRAPHY. By A. I. M. KEULEMANS. Edited by C. G. VERVER. Second Edition. Pp. xxii + 234. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1959. Price $7.50; 60s.

CHEMICAL ANALYSIS OF RESIN-BASED COATING MATERIALS. Edited by C. P. A. KAPPELMEIER. Pp. xxviii + 630. New York and London: Interscience Publishers Inc. 1959. Price $19.50; 147s.

BRITISH CHEMICALS AND THEIR MANUFACTURERS. Pp. ii + 207. London: The Association of British Chemical Manufacturers. 1959. Gratis.

The Directory of the Association of British Chemical Manufacturers.

STUDIES IN CRYSTAL PHYSICS. Original Papers and Papers Reprinted from *Research*, Vol. 11, 1958. By M. A. Jaswon, M.A., Ph.D. Pp. iv + 42. London: Butterworths Scientific Publications. 1959. Price 10s. 6d.

LES RÉACTIONS ÉLECTROCHIMIQUES. By G. CHARLOT, Mme J. BADOZ-LAMBLING and B. TRÉMILLON. Pp. viii + 395. Paris: Masson et Cie. 1959. Price (paper) 6000 fr.; (cloth boards) 6800 fr.

Errata

OCTOBER (1959) ISSUE, p. 576. Equation at bottom of page.

For "$N = \frac{16x^{2''}}{y}$" *read* "$N = \frac{16x^{2}''}{y^{2}}$."

IBID. p. 581, 8th line of text, 1st substance named in Table I and 1st line of text under Table I. *For* "squalene" *read* "squalane."